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.

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*).

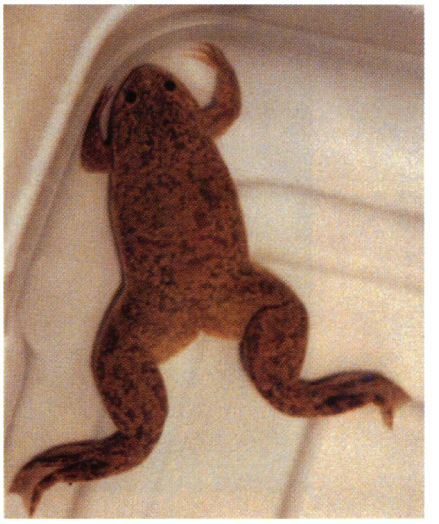
Contaminants in agroecosystems in the western United States. Agricultural needs consume 65% of the available fresh water worldwide (*56*). In the western part of the United States, 80–90% of the water resources are delegated to agricultural uses (*57,58*). Agricultural lands in the West are intensely 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 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.

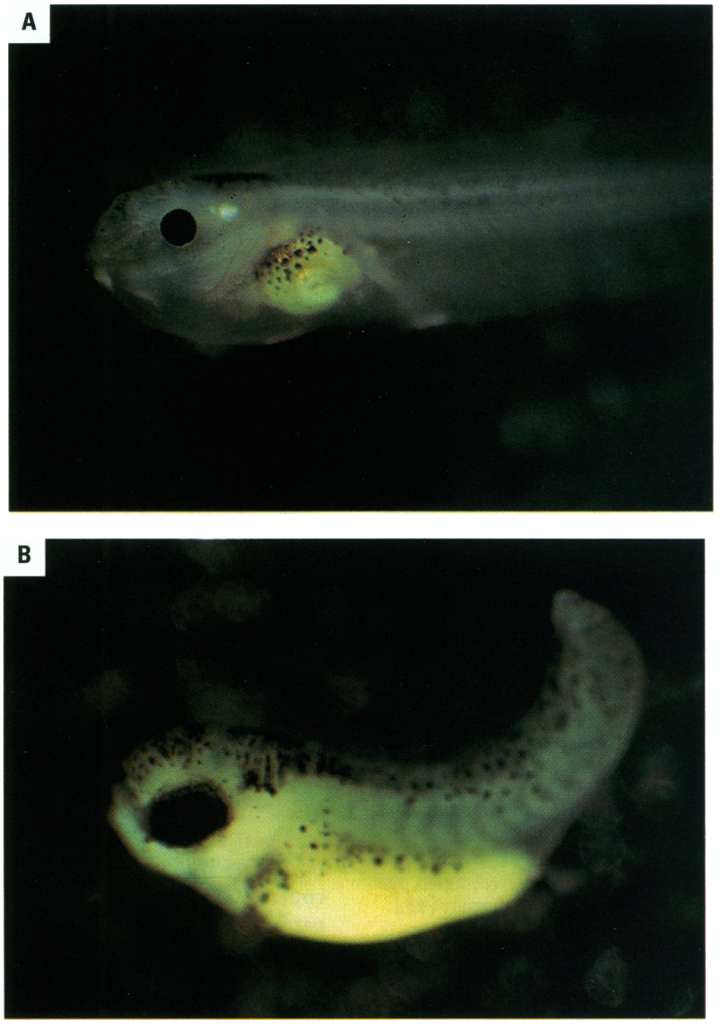


Figure 4. (A) Normally developed embryo at 96 hr (stage 46) and (B) severely malformed embryo of African clawed frog (*Xenopus laevis*) after 96 hr of exposure to agricultural drainwater from the Klamath Basin National Wildlife Refuges.

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

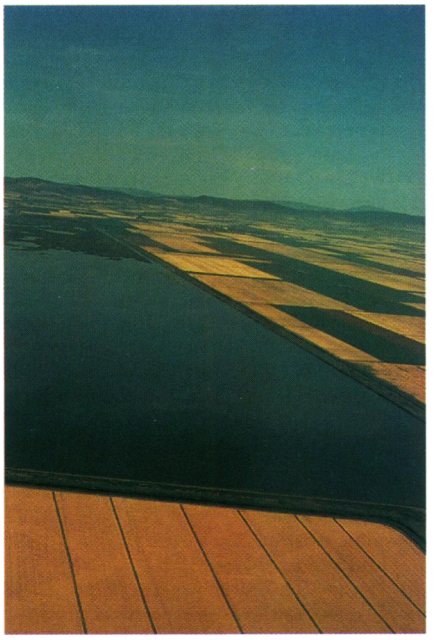


Figure 5. Agricultural fields border the waters of the Tule Lake National Wildlife Refuge on three sides and share water with the lake throughout the growing season. Many other wildlife refuges in the western United States receive irrigation drainwater as a primary source of water.

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.

References

1. Smith RA, Alexander RB, Wolman MG. Water-quality trends in the nation's rivers. Science 235:1609–1615 (1987).
2. Becker CD, Neitzel DA, eds. Water quality in North American river systems. Columbus: Battelle Press, 1992.
3. Hall RJ, Henry PFP. Review: assessing effects of pesticides on amphibians and reptiles: status and needs. Herpetol J 2:65–71 (1992).
4. Chadwick DH. The biodiversity challenge. Special report. Washington DC:Defenders of Wildlife, 1990.
5. Bury RB. Habitat relationships and ecological importance of amphibians and reptiles in streamside management. In: Riparian wildlife and forestry interactions (Raedeke KJ, ed). Seattle:University of Washington Press, 1988; 61–76.
6. Dunson WA, Wyman RL, Corbett ES. A symposium on amphibian declines and habitat acidification. J Herpetol 26:349–342 (1992).
7. Duellman WE, Trueb L. Biology of amphibians. New York:McGraw-Hill Book Company, 1986.
8. Walls SC, Blaustein AR, Beatty JJ. Amphibian biodiversity of the Pacific Northwest with special reference to old-growth stands. Northwest Environ J 8:53–69 (1992).
9. Cohn JP. Salamanders slip-sliding away or too surreptious to count? Bioscience 44:219–223 (1994).
10. Barinaga M. Where have all the froggies gone? Science 247:1033–1034 (1990).
11. Wake DB. Declining amphibian populations. Science 253:860 (1991).
12. Crump ML, Hensley FR, Clark KL. Apparent decline of the golden toad: underground or extinct? Copeia 4:413–420 (1992).
13. Blaustein AR, Wake DB, Sousa WP. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. Conserv Biol 8:60–71 (1994).
14. Allan JD, Flecker AS. Biodiversity conservation in running waters. Bioscience 43:32–43 (1993).
15. Johnson R. Habitat loss and declining amphibian populations. In: Declines in Canadian amphibian populations: designing a national monitoring strategy. Occasional paper no 76 (Bishop CA, Pettit KE, eds). Ottowa, Ontario: Canadian Wildlife Service, 1992;71–76.

16. Pounds JA, Crump ML. Amphibian declines and climate disturbance: the case of the golden toad and the harlequin frog. Conserv Biol 8:72–85 (1994).
17. Mitsch WJ, Gosselink JG. Wetlands. New York:Van Nostrand Reinhold, 1986.
18. Mitchell JG. Our disappearing wetlands. Natl Geogr Mag 182:2–45 (1992).
19. Associated Press. Solution is offered on wetlands protection. Everett Herald, Everett, WA, 17 October 1993.
20. Moyle, PB. Effects of introduced bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin Valley, California. Copeia 1:18–22 (1973).
21. Hayes MP, Jennings MR. Decline of ranid species in western North America: are bullfrogs (*Rana catesbeiana*) responsible? J Herpetol 20:490–509 (1986).
22. Bradford DF. Allotopic distribution of native frogs and introduced fishes in high Sierra Nevada lakes of California: implications of the negative effect of fish introductions. Copeia 3:775–778 (1989).
23. Lewis WM, Helms, DR. Vulnerability of forage organisms to largemouth bass. Trans Am Fish Soc 93:315 (1964)
24. Hine, RL, Les BL, Hellmich BF. Leopard frog populations and mortality in Wisconsin, 1974–76. Technical bulletin no 122. Madison, WI:Department of Natural Resources,1981.
25. Crawshaw GJ. 1992. The role of disease in amphibian decline. In: Declines in Canadian amphibian populations: designing a national monitoring strategy. Occasional paper no 76 (Bishop CA Pettit KE, eds). Ottowa, Ontario: Canadian Wildlife Service, 1992;60–62.
26. Scott NJ. Postmetamorphic death syndrome. Froglog 7:1–2 (1993).
27. Carey C. Hypothesis concerning the causes of the disappearance of boreal toads from the mountains of Colorado. Conserv Biol 7: 355–362 (1993).
28. Blaustein AR, Hokit DG, O'Hara RA. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest Biol Conserv 67:251–254 (1994).
29. Blaustein AR, Hoffman PD, Hokit DG, Kiesecker JM, Walls SC, Hays JB. UV repair and resistance to solar UV-B in amphibian eggs: a link to population declines? Proc Natl Acad Sci USA 91:1791–1795 (1994).
30. Oza GM. Ecological effects of the frog's leg trade. Environmentalist 10:39–42(1990).
31. Phillips K. Tracking the vanishing frogs. New York:St. Martin's Press, 1994.
32. Stephen CE, Mount DI, Hansen DJ, Gentile JH, Chapman GA, Brungs WA. Guidelines for deriving numerical national water quality criteria for the protection of aquatic organisms and their uses. NTIS PB85-227049. Springfield, VA:National Technical Instutite Service, 1985.
33. Power T, Clark KL, Harfenist A, Peakall DB. A review and evaluation of the amphibian toxicological literature. Technical report no 61, Ottowa, Ontario:Canadian Wildlife Service, 1989.
34. Holcombe GW, Phipps GL, Sulaiman AH, Hoffman AD. Simultaneous multiple species testing:acute toxicity of 13 chemicals to 12 diverse freshwater amphibian, fish, and invertebrate families. Arch Environ Contam Toxicol 16:697–710 (1987).
35. Evaluation of aquatic pollutants using fish and amphibian eggs as bioassay organisms. In: Animals as monitors of environmental pollutants. Washington DC:National Academy Press, 1979;108–118.
36. Wesley J, Birge J, Black A, Westerman A. Short-term fish and amphibian embryo-larval test for determining the effects of toxicant stress on early life stages and estimating chronic values for single compounds and effluents. Environ Toxicol Chem 4:807–821 (1985).
37. Birge WJ, Black JA, Westerman AG, Ramey BA. Fish and amphibian embryos—a model system for evaluating teratogenesis. Fundam Appl Toxicol 3:237–242 (1983).
38. Dumont JN, Schultz TW, Buchanan MV, Kao GL. Frog embryo teratogenesis assay: *Xenopus* (FETAX)—a short-term assay applicable to complex environmental mixtures. In: Short-term bioassays in the analysis of complex environmental mixtures III (Waters MD, Sandhu SS, Lewtas J, Claxton L, Chernoff N, Newsow S, eds). New York:Plenum Publishing, 1983;393–405.
39. Goss LB, Sabourin TD. Utilization of alternative species for toxicity testing: an overview. J Appl Toxicol 5:193–217 (1985).
40. Bantle JA, Fort DJ, James BL. Identification of developmental toxicants using the frog embryo teratogenesis assay-*Xenopus* (FETAX). Hydrobiology 189:577–585 (1989).
41. Dawson DA, Fort DJ, Newell DL, Bantle JA. Developmental toxicity testing with FETAX: evaluation of five compounds. Drug Chem Toxicol 12:67–75 (1989).
42. ASTM. Standard guide for conducting the frog embryo teratogenesis assay-*Xenopus* (FETAX). Designation: E1439-91. In: Annual Book of ASTM Standards, vol 11.04. Philadelphia, PA:American Society for Testing and Materials, 1199–1209;1992.
43. Bantle JA, Burton TD, Dawson DA, Dumont JN, Finch RA, Fort DJ, Linder G, Rayburn JR, Buchwalter D, Gaudet-Hull AM, Maurice MA, Turley SD. FETAX interlaboratory validation study: phase II testing. Environ Toxicol Chem 13:1629–1637 (1994).
44. Bantle JA, Burton TD, Dawson DA, Dumont JN, Finch RA, Fort DJ, Linder G, Rayburn JR, Buchwalter D, Maurice MA, Turley SD. Initial interlaboratory validation study of FETAX: phase I testing. J Appl Toxicol 14:213–223 (1994).
45. Kutka F. Low pH effects on swimming activity of *Ambystoma* salamander larvae. Environ Toxicol Chem 13:1821–1824 (1994).
46. Freda J. The influence of acid pond water on amphibians: a review. Water Pollut 30: 439–450 (1986).
47. Pierce BA. Acid tolerance of amphibians. Bioscience 35:239–243 (1985).
48. Freda J, Sadinski WJ, Dunson WA. Long-term monitoring on amphibian populations with respect to the effects of acidic deposition. Water Air Soil Pollut 55:445–462 (1991).
49. Beattie RC, Tyler-Jones R. The effects of low pH and aluminum on breeding success in the frog *Rana temporaria*. J Herpetol 26: 353–360(1992).
50. Sadinski WJ, Dunson WA. A multilevel study of effects of low pH on amphibians on temporary ponds. J Herpetol 26:413–422 (1992).
51. Dawson DA, McCormick CA, Bantle JA. Detection of teratogenic substances in acidic mine water samples using the frog embryo teratogenesis assay-*Xenopus*(FETAX). J Appl Toxicol 5:234–244 (1985).
52. Eilers JM, Brakke DF, Landers DH, Overton WS. Chemistry of lakes in designated wilderness areas in the western United States. Environ Monit Assess 12:3–21 (1989).
53. Turk JT, Spahr NE. Rocky Mountains. In: Acidic deposition and aquatic ecosystems (Charles DF, ed). New York:Springer-Verlag, 1991;471–502.
54. Melack JM, Stoddard JL. Sierra Nevada, California. In: Acidic deposition and aquatic ecosystems (Charles DF, ed). New York: Springer-Verlag, 1991;503–530.
55. Nelson PO. Cascade mountains. In: Acidic deposition and aquatic ecosystems (Charles DF, ed). New York:Springer-Verlag, 1991; 531–563.
56. Postel S. Last oasis. New York:WW Norton and Company, 1992.
57. Reisner M. Cadillac desert. New York:Penguin Books, 1986.
58. Stegner W. Where the bluebird sings to the lemonade springs. New York:Random House, 1992.
59. Connell DW, Miller GJ. Chemistry and ecotoxicology of pollution. New York:John Wiley and Sons, 1984.
60. Van der Leeden F, Troise FL, Todd DK. The water encyclopedia. Second edition. Chelsea, MI:Lewis Publishers, 1990.
61. Pimental D. The dimensions of the pesticide question. In: Ecology, economics, ethics—the broken circle (Bormann FH, Keller SR, eds). New Haven:Yale University Press, 1991; 59–69.
62. Browner CM. The administration's proposals. EPA Journal 20:6–9 (1994).
63. Bishop CA. The effects of pesticides on amphibians and the implications for determining the causes of declines in amphibian populations. In: Declines in Canadian amphibian populations: designing a national monitoring strategy. occasional paper no 76. (Bishop CA, Pettit KE, eds). Ottowa, Ontario:Canadian Wildlife Service, 1992;60–62.
64. Cooke AS. Tadpoles as indicators of harmful levels of pollution in the field. Environ Pollut Ser A Ecol Biol 25:123–133 (1981).
65. Cooke AS. Effects of field applications of the herbicides diquat and dichlobenil on amphibians. Environ Pollut 12:43–50 (1977).
66. Sanders HO. Pesticide toxicities of the western chorus frog (*Pseudacris triseriata)* and Fowler's toad (*Bufo woodhousii fowleri)*. Copeia 2:246–251 (1970).
67. Berrill M, Bertram S, McGillvray L, Kolohon M, Pauli B. Effects of low concentrations of forest-use pesticides on frog embryos and tadpoles. Environ Toxicol Chem 13:657–664 (1994).
68. Snawder JE, Chambers J. Toxic and developmental effects of organophosphorus insecticides in embryos of the South African clawed frog. J Environ Sci Health B 24:205–218 (1989).
69. Birch WX, Prahlad KV. Effects of Nabam on developing *Xenopus laevis* embryos: minimum concentration, biological stability, and degradative products. Arch Environ Contam Toxicol 15:637–645(1986).
70. Mohanty-Hejmedi P, Dutta SK. Effects of some pesticides on the development of the Indian bullfrog *Rana Tigrina*. Environ Pollut Ser A Ecol Biol 24:145–161 (1981).
71. Marian MP, Arul V, Pandian TJ. Acute and chronic effects of carbaryl on survival, growth, and metamorphosis in the bullfrog *(Rana tigrina)*. Bull Environ Contam Toxicol 12: 271–275 (1983).
72. Johnson CR. The effects of five organophosphorus insecticides on thermal stress in tadpoles of the pacific tree frog, *Hyla regilla*. Zool J Linn Soc 59:143–147(1980).
73. Littleton TM. Water quality and fishes of the Lower Klamath and Tule Lake National Wildlife Refuges (MS thesis). Seattle, WA:

University of Washington, 1993.
74. Saiki MK, Jennings MR, Wiedmeyer RH. Toxicity of agricultural subsurface drainwater from the San Joaquin Valley, California, to juvenile chinook salmon and striped bass. Trans Amer Fish Soc 121:78–93 (1992).
75. Schroeder RA, Palawski DW, Skorupa JP. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Tulare lake bed area, southern San Joaquin Valley, California, 1986–87. Water-resources investigations report 88-4001. Sacramento, CA:U.S. Geological Survey, 1988.
76. Presser TA, Ohlendorf HM. Biogeochemical cycling of selenium in the San Joaquin Valley, California USA. Environ Manage 11:805–821 (1987).
77. Sorenson SK, Schwarzbach SE. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Klamath Basin, California and Oregon, 1988–89. Water-resources investigations report 90-4203. Sacramento, California: U.S. Geological Survey, 1991.
78. MacCoy, D. Physical, Chemical, and biological data for detailed study of irrigation drainage in the Klamath Basin, California and Oregon, 1990–1992. Open-file report 93–497. Sacramento, California:U.S. Geological Survey, 1994.
79. Boyer R. Evaluation of water quality in relation to frogs at Klamath Basin National Wildlife Refuges (MS thesis). Seattle:University of Washington, 1993.
80. Thurston RV, Chakoumakos C, Russo RC. Effect of fluctuating exposures in the acute toxicity of ammonia to rainbow trout *(Salmo gairdneri)* and cutthroat trout (*S. clarki*). Water Res 15:911–917 (1981).
81. Thurston RV, Russo RC, Vinofradov GA. Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. Environ Sci Toxicol 15:837–840 (1981).
82. Russo RC, Thurston RV. Toxicity of ammonia, nitrite and nitrate to fishes. In: Aquaculture and water quality. Advances in world aquaculture, vol 3 (Brune DE, Tomasso JR, eds). Baton Rouge, LA:World Aquaculture Society, 1991;58–89.
83. Diamond JM, Mackler DG, Rasnake WJ, Gruber D. Derivation of site-specific ammonia criteria for an effluent-dominated headwater stream. Environ Toxicol Chem 12:649–658 (1993).
84. U.S. EPA. Ambient water quality criteria for ammonia. EPA 440/5-85-001. Washington DC:Environmental Protection Agency, 1985.
85. Jackson W. Nature as the measure for a sustainable agriculture. In: Ecology, economics, ethics—the broken circle (Bormann FH, Keller SR, eds). New Haven, CT:Yale University Press, 1991;43–58.
86. Worster D. The wealth of nature. New York:Oxford University Press, 1993.
87. Jackson, W. At nature's pace. New York: Pantheon Books, 1993.
88. Pimental D, Acquay H, Biltonen M, Rice P, Silva M, Nelson J, Lipner V, Giordano S, Horowitz A, D'Amore M. Environmental and economic costs of pesticide use. Bioscience 42:750–760 (1992).
89. Drost BW, Ebbert JC, Cox SC. Long-term effects of irrigation with imported water on water levels and water quality. Water-Resources Investigations Report 93–4060. Tacoma, WA:U.S. Geological Survey,1993.
90. Grumbine RE. What is ecosystem management? Conserv Biol 8:27–38 (1994).
91. Botkin DB. Discordant harmonies. New York:Oxford University Press, 1990.
92. O'Neill RV, DeAngelis DL, Waide JB, Allen TFH. A hierarchical concept of ecosystems. Princeton, NJ:Princeton University Press, 1986.
93. Carpenter SR, Fisher SG, Grimm NB, Kitchell JF. Global change and freshwater systems. Annual Rev Ecol System 23:119–140 (1992).
94. Vighi M, Chiaudani G. Eutrophication in Europe: the role of agricultural activities. In: Reviews in environmental toxicology 3 (Hodgson E, ed). Amsterdam:Elsevier, 1987; 213–258.
95. Vitousek PM. Beyond global warming: ecology and global change. Ecology 75:1861–1876 (1994).
96. Wilson EO. The diversity of life. New York:Norton, 1992.
97. U.S. Fish and Wildlife Service. Endangered and threatened wildlife and plants. Title 50 code of Federal Regulations, part 17, subpart B, 1993.
98. Leonard WP, Brown HA, Jones LCL, McAllister KR, Storm RM. Amphibians of Washington and Oregon. Seattle, WA:Seattle Audubon Society, 1993.

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