# Possible Interrelations among Environmental Toxicants, Amphibian Development, and Decline of Amphibian Populations


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Many amphibian populations are declining in a number of geographical locations throughout the world. In most cases, the cause or causes are unknown, but are assumed to result from man-made alterations in the environment. We review existing evidence concerning how environmental xenobiotics could contribute to declines of amphibian populations by impacting growth and development of the young. This paper examines the potential roles of toxicants in: *a*) affecting the susceptibility of young to disease; *b*) retarding growth and development of amphibian young; *c*) affecting the ability of larvae to avoid predation; *d*) affecting the development of physiological, morphological, or behavioral processes in a manner that subsequently impairs the ability of the young for future reproduction; and *e*) directly causing mortality of young. These issues are not well studied, and more studies are needed before the roles of environmental xenobiotics in amphibian declines are fully understood. — Environ Health Perspect 103(Suppl 4):13–17 (1995)



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## Introduction

It has become apparent over the last few years that many amphibian populations are declining and that extinction has occurred in a few populations (*1,2*). These phenomena have been documented in a variety of habitats on six continents (*3*). In a few cases, a man-made change in the environment has been extreme enough to be implicated as the direct cause of the death of individuals and, in some cases, extinction of amphibian populations. For instance, spraying of DDT in the forests of Oregon caused mortality in a population of western spotted frogs (*Rana pretiosa*) (*4*); increased UV radiation resulting from atmospheric ozone depletion has been correlated with mortality of amphibian eggs (*5*); and habitat destruction, disturbance and fragmentation are accepted causes of local extinctions (*3*). However, other attempts to demonstrate that man-made causes have been severe enough to cause amphibian mortality have failed (*6*). In most cases, the cause or causes are unknown (*3*). Man-made factors are suspected because of the breadth of geographical areas affected and the rapidity with which these declines are occurring. Environmental toxicants (trace metals, pesticides, industrial chemicals and their byproducts, etc.), UV radiation, introduction of nonnative predators (usually fish) or competitors, and acid rain have all been suggested as potential causes, acting singly or synergistically (*7*). It is likely that no single factor or group of factors has been the causative agent throughout the world; each locality may have its own particular cause or causes.

Environmental change could cause population declines in a number of different ways. A lethal change in the environment can kill a population—some or all age classes including eggs and larvae—directly or indirectly by suppressing the immune system and allowing subsequent infection with opportunistic pathogens. Or, population size could be reduced by reproductive impairment. Reproductive success could be impaired by environmental interference with adult reproductive function (inhibition of breeding behavior, manufacture of gametes, or of fertilization) or by disruption of development and growth of the young. While each of these possibilities merits serious attention, this paper will focus on the evidence supporting the hypothesis that environmental toxicants could contribute to amphibian declines by affecting growth and development of the young.

Reproduction is a vulnerable period in the life cycle of amphibians, even in the absence of environmental toxicants. Adults are more at risk from predation when they congregate around breeding ponds than after they have dispersed, and few of the hundreds to thousands of eggs laid by each species normally survive to become breeding adults. For instance, only 4%, 4.4% and 3.3% of wood frog (*Rana sylvatica*), spotted frog (*Rana pretiosa*), and tiger salamander (*Ambystoma tigrinum*) eggs, respectively, survive to metamorphosis (*8*). Over a 5-year period, survivorship of spotted salamanders (*Ambystoma maculatum*) between the egg stage and dispersal of newly metamorphosed young in a pond in Massachusetts varied from 1 to 12.6% (*9*). Although this mortality has been assumed to be naturally caused by predators, by desiccation of eggs as breeding ponds evaporate, by flooding of the breeding pond, or by low temperatures (*8,9*), the role of environmental toxicants in mortality of young amphibians in the field is less understood. Environmental toxicants may interfere with amphibian growth and development in a number of ways: *a*) sublethal concentrations of toxicants may indirectly cause death by promoting susceptibility of eggs and larvae to pathogenic organisms and disease; *b*) sublethal levels of toxicants may indirectly affect survival by retarding growth and metamorphosis with the result that larvae are unable to metamorphose and


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depart breeding ponds at the appropriate time; *c*) sublethal levels may inhibit the ability of larvae to avoid predators; *d*) toxicants that have estrogenic, antiestrogenic, thyroid-disrupting, androgenic, or antiandrogenic properties may either impair or totally inhibit future reproduction by disrupting developmental processes; or *e*) lethal concentrations of toxicants might directly cause mortality of the eggs, larvae, or metamorphosing individuals. We will review existing evidence on these five possibilities and point out areas in which more data are needed before firm conclusions can be reached.

## Potential Role of Toxicants in Increasing Susceptibility of Young to Disease

A recent hypothesis suggests that amphibian mortality in the field does not necessarily have to be caused by severe environmental changes that are directly lethal (*2*). Sublethal environmental changes, acting singly or synergistically, could stress larval or postmetamorphic amphibians sufficiently that their immune systems become compromised; infection by opportunistic pathogens is followed by death (*2*). This proposal is based on observations that local die-offs and even extinctions of amphibian populations have been associated with infections of opportunistic bacteria such as *Aeromonas hydrophila* (*1,2,10*). These bacteria are ubiquitous in fresh water, present on the skin and in the digestive tracts of healthy organisms, and apparently, successfully attack immunosuppressed individuals (*11*). Laboratory studies indicate that immunosuppression can be induced by injection of corticosterone (one of the hormones associated with responses to stress) or exposure to heavy metals, certain pesticides or industrial chemicals, and cold (*12–14*). Therefore, it is possible that sublethal changes in one or more of these factors can cause immunosuppression, leading to infection and subsequent death in amphibian populations in the field. This hypothesis is currently being tested.

Since resistance to pathogens results from a complex, multifaceted immune system that gradually develops during ontogeny, amphibian eggs, larvae, and metamorphosing, individuals might be more vulnerable to environmentally influenced disruption of immune function than adults. Although many existing reports of die-offs due to bacterial infection document only mortality of adults and subadults (*2*) or mortality of both adults and larvae (*10*), a few observations raise the possibility that larvae or particular size classes of newly metamorphosed individuals might be more susceptible to disease than adults. For instance, infection of *Aeromonas hydrophila* was more prevalent in larval than adult leopard frogs (*Rana pipiens*) in a population in Minnesota (*15*). Infection by *Aeromonas hydrophila* caused mass mortality of larval wood frogs (*Rana sylvatica*) in ponds in Rhode Island, but adults in the same pond appeared unaffected (*16*). Most smaller frogs in one population of yellow-legged frogs (*Rana muscosa*) in the Sierra Nevada succumbed to an infection of *Aeromonas hydrophila* or *Enterobacter* sp., while larger frogs survived (*1*). Most newly metamorphosed *Rana yavapiensis* were killed during an epidemic of *Aeromonas hydrophila*, whereas most adults in the same pond near Phoenix, Arizona, survived (M Sredl, unpublished observation). Finally, two mass die-offs of tiger salamander (*Ambystoma tigrinum*) larvae and newly metamorphosed young in montane ponds of Colorado were associated with the presence of *Aeromonas hydrophila* and *Acinetobacter* sp., but adult carcasses were not found (CJ Bryant and C Carey, unpublished observation). It is unclear whether the salamander adults had left the pond before the onset of the bacterial epidemic, or whether they were unaffected by disease. Could developmental differences in amphibian immune function result in differential abilities of larvae, individuals undergoing metamorphosis, and adults to resist disease when subjected to environmental stress?

Considerable diversity exists among amphibians in body form, breeding characteristics, and habitats (ranging from completely aquatic to completely terrestrial). The class Amphibia is comprised of at least 4000 living species grouped into three orders: salamanders (Caudata), caecilians (Gymnophonia), and frogs and toads (Anura) (*17*). Important differences in structure and function of the immune system may well exist in each taxonomic group, but research has focused on relatively few species. The immune system of the African clawed frog (*Xenopus laevis*) has been studied intensively, and immune function in a few species of *Rana*, *Ambystoma*, and *Bufo* has received considerable attention, but the most frequently studied species in these genera account for less than 1% of the total species in the Amphibia (*17*). No information is available concerning the immune system of the vast majority of species. Therefore, the generalizations given below concerning the amphibian immune system derive from the specific findings from studies on *Xenopus laevis*, and possibly also from those on *Rana*, *Ambystoma* and *Bufo*.

Amphibian eggs are enclosed in a jelly capsule at laying. The jelly capsule of *Xenopus* has three layers, each of which is composed of different amounts of polysaccharides and proteins (*18*). The fact that the composition of each layer differs from that of the others suggests that each layer may have a different function. Little information exists on whether the jelly capsule or the egg is provisioned with anti-pathogenic defenses or whether the jelly capsule simply functions as a mechanical barrier. However, the observations that *Bufo* eggs develop fungal infections following handling (C Carey, unpublished observation) suggest that eggs and embryos are protected until hatching by one or more defense mechanisms in intact and undisturbed jelly. Recent observations show that fungal infections (*Saprolegnia ferax*) of eggs resulted in almost complete reproductive failure of one population of boreal toads (*Bufo boreas boreas*) in the Cascades of Oregon (*19*). It is unclear whether such infections result from recent introduction of fish carrying the fungus into breeding areas of these toads, or whether environmental factors are reducing protective qualities of the jelly capsule against such pathogens.

The tissues that play important roles in amphibian immunological responses are thymus, spleen, bone marrow, kidney, liver, and various aggregations of lymphoid cells that resemble mammalian lymph nodes (*20*). The relative role and importance of each tissue differ between larvae and adults. Ventral and dorsal cavity bodies play an important lymphogenic role in *Xenopus* larvae but disappear after metamorphosis. Lymphoid nodules are present in the intestine of adults but are absent in larval *Xenopus*. The bone marrow of *Xenopus* develops as limbs develop and becomes calcified after metamorphosis. The liver of *Xenopus* retains lymphopoietic function throughout life, but this function disappears after metamorphosis in other anurans, which have been examined. The thymus begins development around day 3 after fertilization (about stage 40 of development), grows to contain roughly $1\times10^6$ lymphocytes within 45 days (stage 58), shrinks during metamorphosis, and then grows during the first 2 to 3 months after metamorphosis to contain about 1 to $3\times10^7$ cells; it undergoes a final regression at the onset of sexual maturity (*21*).

Primordial spleen cells appear about 12 to 14 days after fertilization in *Xenopus;* once functional, the spleen continues as a lymphopoietic organ throughout life (*21*).

Immune systems of *Xenopus* larvae acquire the ability to recognize and destroy transplants of foreign tissue within about 12 days (stage 49) after hatching, but full response to allografts does not occur until well after metamorphosis (21). B-cells, which produce antibodies, begin maturation in the liver within 5 days of hatching (stage 45), and the first antibodies (IgM and IgY) appear in the serum by days 10 to 13 (stages 49–51). *Xenopus* larvae begin recognition of foreign antigens by days 19 to 20 (stage 51–52). During metamorphosis, the larval antibody repertoire is completely replaced by adult antibodies. These observations provide support for the idea that immune systems of young may be more vulnerable to environmental disruptions of immune function than those of adults because the full suite of defenses is not completely developed until well after metamorphosis (*21*).

## Potential Role of Toxicants in Retarding Growth and Development of Young Amphibians

Some larval amphibians complete development and metamorphosis within their first summer, while others overwinter as larvae and go through metamorphosis during their second summer (*22*). In some species such as bullfrogs (*Rana catesbeiana*), larvae overwinter at least 1 year in the northern parts of their range in the United States, but long growing seasons in the southern parts of their distribution foster metamorphosis of larvae in their first summer (*23*). The ability to overwinter in a larval form undoubtedly requires special adaptations that maximize survival in aquatic surroundings, such as the ability to tolerate anoxia (*24*). Larvae that typically complete metamorphosis by the end of their first summer undoubtedly survive their first winter with a different suite of specializations than those overwintering as larvae. These specializations, such as the ability to tolerate freezing, would foster survival in a variety of environments (soil, subterranean cavities, or under water). If growth and development of larvae that commonly complete metamorphosis their first summer and overwinter as metamorphosed individuals are delayed by toxicants so that they are forced to overwinter as larvae, they may not survive the winter because their population or species may not have evolved the specializations that promote winter survival in the larval form.

Retardation of growth may have other negative effects. Rapid growth has a selective benefit for many amphibian species because they are subjected to size-specific predation. Rapidly growing larvae suffer a lower cumulative risk of death because they spend less time in smaller, more vulnerable stages than do slower growing ones (*25*).

A recent experiment documents that exposure to various combinations of low pH and aluminum (as $AlCl_3 \cdot 6H_2O$) retards growth and development of green treefrog (*Hyla cinerea*) tadpoles (*26*). Low pH was tested in conjunction with aluminum because heavy metals tend to be leached out of soils in contact with acidic water (*27*). Body length of larvae maintained for 96 hr at lower pH (5.5 and 4.5) and higher concentrations of aluminum (up to 400 µg/l ) was significantly reduced compared to controls (pH 7.0, no aluminum), whereas length of tadpoles maintained at lower pH alone was not significantly affected.

Another example of the relation between exposure to toxicants and amphibian growth is provided by a study on the effect of freshwater petroleum contamination on hatching success and growth rates of young *Hyla cinerea.* The findings indicated that while hatching success was not significantly impacted by exposure to 10, 55, and 100 mg/l of crankcase oil, growth rates of larvae exposed to higher concentrations of oil were significantly retarded (*28*).

It is unknown exactly how pH, aluminum, or various other pollutants retard growth in these tadpoles; interference with food acquisition, food digestion, uptake of digestive nutrients, or synthesis of new tissues are only a few of many possibilities. Acute exposure to low pH (2.5–4.0) causes a reduction in sodium influx and acceleration in sodium efflux in leopard frog (*Rana pipiens*), bullfrog (*Rana catesbeiana*), and green frog (*Rana clamitans*) tadpoles (*29*). The energetic costs of active transport necessary to counteract changes in sodium flux at low, but not lethal, pH could potentially detract from energy available for growth. While more studies testing single and synergistic effects of pH and environmental toxicants are needed, these results support the contention that environmental change could lead to decreases in size of amphibian populations by retarding normal growth and development.

Amphibians undergo metamorphosis at a small fraction of adult body size and grow substantially after metamorphosis. For instance, an average ranid frog completes metamorphosis at a larval mass corresponding to about 6% of adult body mass. Postmetamorphic growth typically accounts for 80 to 99% of the adult body mass in anurans (*22*). The effect of environmental toxicants on postmetamorphic growth of any amphibian species is unknown.

## Potential Roles of Toxicants in Affecting Larval Ability to Avoid Predation

Predation can be a major cause of larval mortality in amphibian populations (*30*). Insect larvae, fish, snakes, birds, and other amphibians are probably the major predators on amphibian larvae. Larvae that are slow swimmers are more frequently predated upon than more rapid swimmers (*31*). Exposure of amphibian larvae early in development to high, but sublethal, levels of toxicants causes deformities in the body or tail that clearly impact swimming ability (*32*). Even when toxicant levels do not result in deformities, either because toxicant concentrations are low or because exposure occurred after larvae had passed certain critical stages in development, swimming ability may still be compromised. A recent study on the effects of low pH and aluminum concentrations on swimming performance and susceptibility to predation indicated that *Hyla cinerea* larvae exposed to pH of 4.5 and 100, 200, or 400 µg/l Al exhibited reduced swimming performance compared to controls (pH 4.5, 0 µg/l Al), even when differences in body length were taken into account (*26*). Tadpoles exposed to pH 4.5 and 150 µg/L Al were more susceptible to predation by dragonfly larvae (family Libellulidae) than controls (pH 7.0, 0 µg/l Al). The dragonfly larvae may have eaten more experimental than control tadpoles because they were easier to capture at a slower swimming speed, because their relatively smaller size made it easier for the dragonfly larvae to eat, or because dragonflies had to eat more of them in order to fill their nutritional needs (*26*). The mechanism by which low pH and aluminum impair swimming performance is unknown.

Reduction in swimming performance is not necessarily the only way in which toxicants increase the susceptibility of amphibian larvae to predation. Exposure to certain environmental toxicants causes a period of hyperactivity in amphibian larvae. *Rana temporaria* tadpoles treated with DDT swam rapidly, twisted their bodies, and

lashed their tails prior to becoming moribund and dying (*33*). Warty newts (*Triturus cristatus*) prey on significantly more of the hyperactive tadpoles than slow swimming tadpoles (*33*).

## Potential Role of Toxicants in Disruption of Developmental Processes Leading to Alteration of Reproductive Potential

It is now firmly established that many man-made chemicals (pesticides, industrial chemicals and by-products, heavy metals, etc.) disrupt endocrine systems of wildlife (*34*). The fact that many of these compounds either mimic the effects of estrogen or androgens or have anti estrogen or anti-androgen effects has received a great deal of attention because of the serious consequences for reproduction; sexual behavior, fertility, development of the gonads and sexual organs, etc. can all be negatively affected by exposure of young to these compounds. Unfortunately, very few studies yet exist on whether reproduction of amphibian populations in the wild has been affected by environmental toxicants. It is interesting to speculate that members of the last remaining population of the endangered Wyoming toad (*Bufo hemiophrys baxteri*) may have been negatively impacted by endocrine-disrupting chemicals. Males exhibited little clasping behavior at the appropriate breeding time in spring and hatchability of the few clutches resulting from amplexus was very low (A Anderson, unpublished observation). In another study, *Ambystoma tigrinum*, inhabiting a lagoon polluted with secondary domestic sewage and perylene (a component of jet fuel), were reproductive as neotenes, rather than as metamorphosed adults. Metamorphosis may have been inhibited by environmental pollutants. Furthermore, the majority of the neotenes in the polluted lagoon also suffered skin lesions; 84% were neoplasms. The effects of pollutants were not permanent because, when animals were transferred out of the polluted lagoon into an adjacent, relatively unpolluted pond, they metamorphosed within 6 to 9 months, and many of these lesions regressed (*35*).

It has been assumed that tissue concentrations of toxicants below levels at which they can be detected by chemical analysis are safe (*36*). However, because endocrine-disrupting toxicants can have effects at tissue levels well below detectable levels, toxicants designated as safe should not be considered to be free of endocrine-disrupting effects until proven otherwise.

## Potential Roles of Toxicants as Lethal Agents for Young Amphibians

A wealth of data exists on tolerance levels of amphibian larvae to various environmental toxicants. The acute toxicity effects of over 211 different pollutants have been studied on at least 45 different species of amphibians, and effects of at least 54 different substances have been studied in field applications (*37*). For instance, reproductive success in a population of *Rana temporaria* was reduced after spraying of atrazine nearby (*38*), and northern cricket frogs (*Acris crepitans*) died in a stream adjacent to a cotton field in which DDT had been applied (*39*). While a variety of results have been obtained because of the number of species, life stages, and techniques used, the literature suggests that adult and larval amphibians are not necessarily more sensitive to chemicals than are other land or aquatic vertebrates (*37*). However, it is interesting to note that anurans are remarkably more resistant to cholinesterase inhibitors than are other vertebrates, including urodeles (*37*).

Despite the importance of the large amount of information gathered in laboratory and field studies, the latter of which have mostly been conducted in agricultural areas or heavily polluted areas, we are lacking much information concerning toxicant exposure of amphibians in pristine areas of the western United States where populations are declining rapidly. Since deposition of airborne pollutants is greatest in montane areas where highest levels of snowfall occur, animals living in montane habitats are likely to be exposed to higher levels of toxicants, especially during snowmelt, than are those living at lower altitudes (D Haddow, personal communication). Exposure information is badly needed; we need baseline data on tissue levels of toxicants in amphibians and on toxicant concentrations in sediments, water, and prey in a wide variety of habitats. The time frame remains to be determined within which application of various toxicants would prove lethal in the field. The relative vulnerability of eggs, larvae, and adults of various amphibian species to different toxicants in the field has yet to be established. Therefore, estimation of the extent to which environmental xenobiotics have contributed directly to amphibian population declines is extremely difficult to determine at this time.

## Summary

In most cases, causes of amphibian population declines are unknown. This paper has reviewed several ways in which these declines could have been caused by environmental toxicants, but critical data are lacking in most instances. Coordinated studies in both the field and laboratory are needed to establish whether causal relations exist between levels of environmental toxicants and the demise of amphibians.

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