

## Emerging Infectious Diseases and Amphibian Population Declines

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We review recent research on the pathology, ecology, and biogeography of two emerging infectious wildlife diseases, chytridiomycosis and ranaviral disease, in the context of host-parasite population biology. We examine the role of these diseases in the global decline of amphibian populations and propose hypotheses for the origins and impact of these panzootics. Finally, we discuss emerging infectious diseases as a global threat to wildlife populations.

Emerging infectious diseases have been reported increasingly as causes of death in free-living wild animals (1). These diseases are a particular threat to wildlife species whose population, habitat, or range has been diminished or artificially manipulated to promote species survival (e.g., captive breeding, translocation, and release programs) (2-4). An early example of an emerging disease panzootic was the introduction of rinderpest in African domestic cattle in 1889 (5). More recently, epizootics and panzootics of wildlife have been increasingly reported in terrestrial (1) and marine (6) habitats and are probably underreported (1,4,7-9). Recent advances in theoretical and experimental host-parasite ecology have demonstrated a major role for infectious agents in the population biology of wild animals (10,11). We discuss recent data on two newly emerging infectious diseases of amphibians and, by reference to host-parasite ecology, propose hypotheses to explain their origin and impact.

### Amphibian Population Declines

Global declines in amphibian population are perhaps one of the most pressing and enigmatic environmental problems of the late 20th century

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(12-19). While some declines are clearly due to habitat destruction, others are not associated with obvious environmental factors. Causal hypotheses include the introduction of predators or competitors, increased ultraviolet (UV-B) irradiation, acid precipitation, adverse weather patterns, environmental pollution, infectious disease, or a combination of these. Transdermal water uptake and gaseous exchange and a biphasic life cycle are important aspects of amphibian biology. These factors led to the hypothesis that amphibians act as sentinels for global environmental degradation (12,18). However, this role has yet to be demonstrated, and many causal factors may be present (12,19,20).

Of particular concern are population declines in ecologically pristine areas, such as the montane tropical rain forests of Australia and Central America, where human impact from agriculture, deforestation, or pollution is thought to be negligible. Here, long-term data demonstrate recent and catastrophic amphibian population declines, often resulting in the complete loss of amphibian species (local extinction of multiple species) from large swaths of habitat (20-25). These declines include the disappearance and presumed extinction of the recently discovered golden toad (*Bufo periglenes*) of Costa Rica (23) and as many as seven Australian amphibian species, including two species of gastric-brooding

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frog (*Rheobatrachus* spp.) (20). These data, along with recent findings of amphibian mass deaths in these areas, suggest that such local extinctions are not normal population fluctuations or metapopulation dynamics.

Investigations in Australia, the United Kingdom, and North and Central America

(26-31) have repeatedly found two diseases as the causes of amphibian mass deaths globally (Table 1): chytridiomycosis in the rain forests of Australia and Central and South America and some parts of North America (28-32) and iridoviral infections in the United Kingdom, the United States, and Canada (26,27,33-36). Both

Table 1. Mass deaths caused by chytridiomycosis and ranaviral disease in wild populations of amphibians

	Locality and date of mass deaths	Species affected and impact <sup>a</sup>	References
Chytridiomycosis	E. & S. Australia (1993-1999) <sup>b</sup>	Multiple montane rain forest and temperate species. Mass deaths, local extinctions, population declines. Near-extinction of <i>Taudactylus acutirostris</i> . Hypothesized link with global extinction of two species of gastric brooding frog ( <i>Rheobatrachus</i> spp.).	28-30
	W. Australia (1998-1999) <sup>c</sup>	Multiple species, predominantly the western green (or motorbike) frog ( <i>Litoria moorei</i> ). Mass deaths, population declines.	29,31
	Costa Rica and Panama (1994-99)	Multiple montane rain forest species. Mass deaths, local extinctions, population declines. Hypothesized link with global extinction of golden toad, <i>Bufo periglenes</i> .	20,23, 28,29
	Ecuador (1999)	Montane rain forest <i>Atelopus</i> species, <i>Telmatobius niger</i> , and <i>Gastrothecus pseustes</i> . Unknown impact.	29
	Arizona (1996-1997)	Leopard frog ( <i>Rana yavapiensis</i> & <i>R. chiricahuensis</i> ). Mass deaths.	29
	S. Arizona (1999)	Leopard frog ( <i>Rana</i> sp.). Mass deaths.	31,32
	Colorado (1999)	Boreal toad ( <i>Bufo boreas</i> ). Mass deaths.	<sup>d</sup>
	Colorado (1970s)	Leopard frog ( <i>Rana pipiens</i> ). Mass deaths.	32 <sup>d</sup>
	Sierra Nevada, California (1970s)	Yosemite toad ( <i>Bufo canorus</i> ). Mass deaths.	32 <sup>e</sup>
	Ranaviral disease	United Kingdom (1992-1999) <sup>f</sup>	Common frog ( <i>Rana temporaria</i> ). Mass deaths, possibly population declines.
Arizona (1995)		Sonoran tiger salamander ( <i>Ambystoma tigrinum stebbinsi</i> ). Mass deaths in this endangered species.	27
N. Dakota (1998)		Tiger salamander ( <i>A. tigrinum</i> ). Mass deaths.	35
Maine (1998)		Tiger salamander ( <i>A. maculatum</i> ). Mass deaths.	35
Utah (1998)		Tiger salamander ( <i>A. tigrinum</i> ). Mass deaths.	35
Saskatchewan, Canada (1997)		Tiger salamander ( <i>A. tigrinum diaboli</i> ). Mass deaths.	36

<sup>a</sup>Mass deaths did not occur in all cases of wild amphibians infected by chytridiomycosis. *Bufo americanus* from Maryland and *Acris crepitans* from Illinois have been found infected with chytridiomycosis without observed deaths (37,38). In Australia, chytridiomycosis has been reported from small numbers of amphibians without evidence of clinical signs or deaths in both upland and lowland species (R. Speare, L. Berger, unpubl. obs.).

<sup>b</sup>Retrospective studies have identified chytridiomycosis as the cause of death in wild frogs in five Australian states from as early as 1989 (29).

<sup>c</sup>This recent outbreak was more than 2,000 km from the closest recorded chytridiomycosis-linked amphibian die-offs (31). It is thought that chytridiomycosis may now be enzootic in many areas of Australia, but still in the process of spreading to naïve populations. A role for chytridiomycosis in other recent W. Australian declines is suspected due to similarities in the pattern of declines and presence of the *Batrachochytrium* carcasses from W. Australia since 1992.

<sup>d</sup>D.E. Green, unpubl. obs.

<sup>e</sup>D.E. Green, unpubl. obs. Historically collected specimens recently examined histologically revealed chytridiomycosis as a contributing factor to the cause of death in 2 of 12 animals.

<sup>f</sup>A.A. Cunningham, unpubl. obs.

diseases have been classified as emerging (1). The parasitic infection recently implicated as the cause of amphibian deformities in North America has not been associated with mass deaths or population declines (31).

### Chytridiomycosis—an Emerging Panzootic Fungal Disease of Amphibians

Chytridiomycosis is a fungal disease first described in 1998 from moribund and dead adult amphibians collected at sites of mass deaths in Australia and Panama from 1993 to 1998 (28). Here, long-term ecologic study sites reported catastrophic amphibian population declines in Big Tableland, Queensland (39,40), and Fortuna and Cerro Pando, Panama (24,25,28). No significant pathogens were found on routine parasitologic, bacteriologic, mycologic, or virologic examinations of tissue samples (28). Fresh skin smears and histologic sections of the epidermis, however, consistently contained large numbers of developing and mature sporangia of a new genus of chytrid fungus (phylum Chytridiomycota) (Figures 1, 2). Sporangia were also present within the keratinized mouthparts, but not the epidermis, of sympatric tadpoles (tadpoles lack epidermal keratin) (28,29). No significant morphologic differences between chytrids infecting Australian and Central American amphibians were found by transmission electron microscopy, and the pathogen was identified as a member of the order Chytridiales by analysis of zoospore ultrastructure and 18S rDNA sequence data (28). Chytrids are ubiquitous fungi that develop without hyphae and are found in aquatic habitats and moist soil, where they degrade cellulose, chitin, and keratin (41). Parasitic chytrids mainly infect plants, algae, protists, and invertebrates (41); the amphibian pathogen is the only example of a chytrid parasitizing vertebrates (28).

Clinical signs of amphibian chytridiomycosis include abnormal posture, lethargy, and loss of righting reflex. Gross lesions, which are usually not apparent, consist of abnormal epidermal sloughing and (more rarely) epidermal ulceration; hemorrhages in the skin, muscle, or eye; hyperemia of digital and ventrum skin, and congestion of viscera (29). Diagnosis is by identification of characteristic intracellular flask-shaped sporangia and septate thalli within the epidermis (Figures 1, 2).

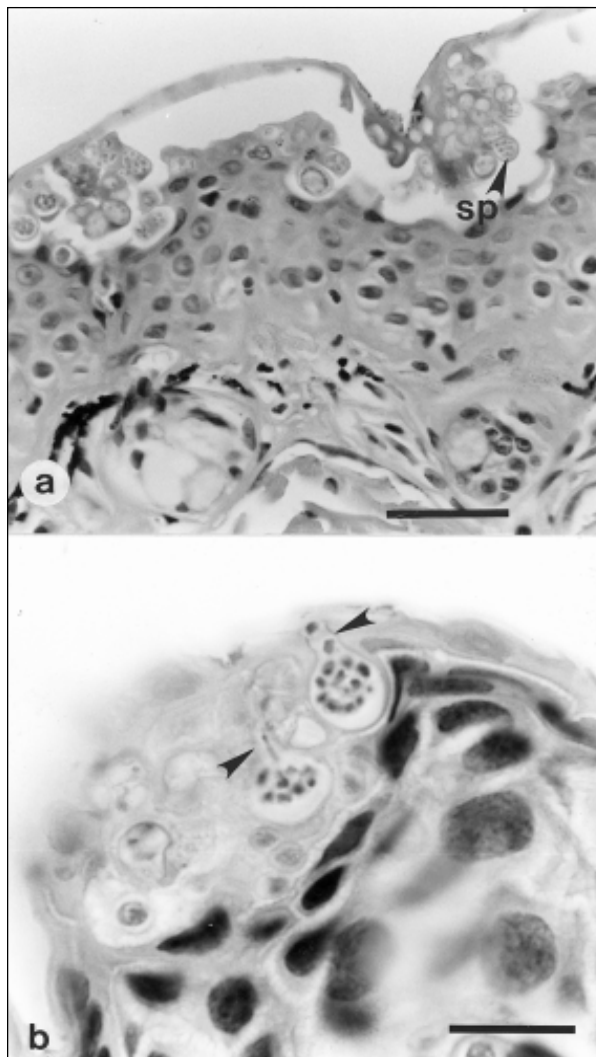


Figure 1a: Ventral abdominal skin of *Bufo haematiticus* from western Panama. The superficial keratinized layer of epidermis (*stratum corneum*) contains numerous intracellular spherical-to-ovoid sporangia (spore-containing bodies) of *Batrachochytrium* sp. The mature sporangia (sp, arrows) are 12-20  $\mu\text{m}$  ( $n = 25$ ) in diameter and have refractile walls 0.5-2.0  $\mu\text{m}$  thick. Most sporangia are empty, having discharged all zoospores, but a few sporangia contain two to nine zoospores. This *stratum corneum* is markedly thickened adjacent to groups of parasitized cells and in some cases, the superficial layer has become detached. No chytrids are present in the *stratum spinosum*, *stratum basale*, dermis, dermal glands, and blood vessels. Note the absence of hyphae and lack of an inflammatory cell response in the deeper layers of epidermis and the dermis. Hematoxylin and eosin stain. Bar = 35  $\mu\text{m}$ . 1b. Ventral skin of upper hind limb of *Atelopus varius* from western Panama. Two sporangia containing numerous zoospores are visible within cells of the *stratum corneum*. Each flask-shaped sporangium has a single characteristic discharge tube (arrow) at the skin surface. Exiting zoospores are visible in the discharge tubes of both sporangia. Hyperkeratosis is minimal in this acute infection. Tissues were fixed in neutral-buffered 10% formalin, paraffin-embedded, sectioned at 6  $\mu\text{m}$  thick and stained with hematoxylin and eosin. Bar = 35  $\mu\text{m}$ .

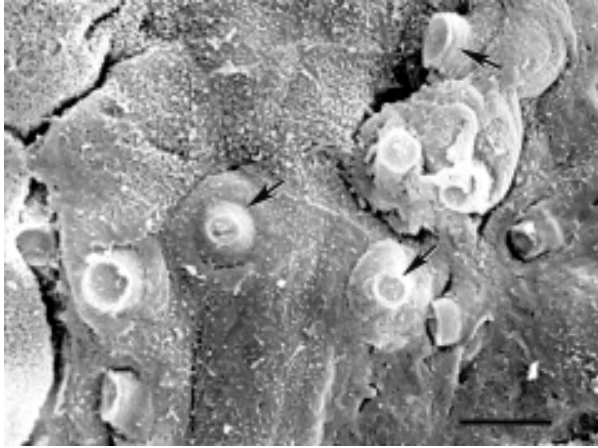


Figure 2. Scanning electron micrograph of digital skin of a wild frog (*Litoria lesueuri*, from Queensland, Australia) that died of cutaneous chytridiomycosis. Many cells within this area of the superficial layer of the epidermis contain mature sporangia, and unopened discharge tubes are visible protruding through infected cells. The skin was fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated, critical-point-dried, sputter coated with gold, and examined with a JEOL JSM 840 scanning electron microscope at 5 kV. All specimens are from animals that were naturally infected and died due to chytridiomycosis in montane rain forest regions of Panama and Australia. Bar = 5  $\mu$ m. Photo courtesy of L. Berger, reprinted with permission from A. Campbell (29).

Its occurrence solely in keratinized tissue suggests that the chytrid uses amphibian keratin as a nutrient. A hyperkeratotic and hyperplastic response of the epidermis to infection (restricted to the *stratum corneum* and *stratum granulosum*) usually coincides with the immediate location of chytrid developmental stages. Inflammatory cell response is negligible. An isolate cultured from captive dendrobatid frogs has recently been used to fulfil Koch's postulates as a fatal pathogen of frogs and has been described as a new genus and species, *Batrachochytrium dendrobatidis* (42). Three mechanisms by which chytridiomycosis causes death have been proposed (28): 1) epidermal hyperplasia impairs essential cutaneous respiration or osmoregulation; 2) a fungal toxin is absorbed systemically (although a lack of clinical signs in infected larvae suggests otherwise); and 3) these factors are combined (28,37).

Comparison of histologic, ultrastructural, and 18s rDNA sequence data indicates that the chytrids found in wild Australian (28) and

captive Central American (T.Y. James, D. Porter, J.E. Longcore, pers. comm.) amphibians belong to the genus *Batrachochytrium*, are probably conspecific, and form a distinct monophyletic clade within the Chytridiales.

### Emergence of Chytridiomycosis

Retrospective histologic surveys of museum specimens of montane, riparian anurans from protected sites in Central America and Australia, conducted 1 to 10 years before the population declines, showed no evidence of chytrid infection, which suggests that chytridiomycosis has recently emerged on two continents (28). The relatively synchronous discovery of chytridiomycosis in Australia and Central America in association with amphibian population declines is striking. The data suggest that *Batrachochytrium* 1) may be endemic to these regions and the amphibian deaths and declines attributed to it have only recently been discovered; 2) may be endemic and has recently become pathogenic (e.g., through an increase in the organism's prevalence or virulence, or a decrease in the host's defenses), or 3) may have been introduced recently into these geographic regions and is now parasitizing novel host species.

The pattern of amphibian deaths and population declines associated with chytridiomycosis is characteristic of an introduced virulent pathogen dispersing through a naïve population (7,10,39). In Australia, a distinct geographic and temporal progression in population declines has occurred (20), moving northward at a mean rate of 100 km per year (39). In Central America, a progression from northern Costa Rica to western Panama occurred from 1996 to 1998 (24,25). The uneven progression of declines in Australia (40) may reflect gaps in surveillance. Small-scale irregularities, however, characterize the epidemiology of many pandemics (43,44), within which individual epidemics progress at different rates in different areas. In amphibian populations, unevenness may be due to differences in ecologic factors (e.g., population density, habitat, age structure); differences among pathogen strains; stochastic factors, such as the time of introduction; or a combination of these factors. In Australia and Central America, population declines have been catastrophic, occurring over a few months, with dramatic population loss and high rates of adult deaths (20,24,25,28,39,40,45).

Such high depopulation rates are characteristic of introduced virulent pathogens (10). Conversely, in coevolved host-pathogen relationships, a degree of herd immunity to the pathogen and lower virulence (infectivity and death rates) are normally observed. The low host specificity of amphibian chytridiomycosis (more than 30 species of wild amphibians from seven families in Central America and Australia [28,29]) also suggests that the disease was not enzootic in those montane rain-forest populations. Ability to infect a range of host species is a characteristic of many invading pathogens (10) and is less common in endemic microparasites that have coevolved with their hosts.

The most parsimonious hypothesis for the origin of chytridiomycosis panzootics in Australia and Central America is the introduction of disease into populations of previously unexposed amphibians. Introduction of pathogens, termed pathogen pollution (1), is increasingly recognized as a significant threat to global biodiversity (1,6,46) and forms an integral part of human history (10,47). There are precedents for the introduction of fungal pathogens (including chytrid parasites) that cause high death rates (41,48). Mechanisms by which wildlife pathogens can be introduced are common; for example, a consequence of the increasing mobility of humans is the global translocation of wildlife, plants, soil, and ballast water (1,4,49,50). Freshwater fish and amphibians are also transported globally. In Australia, chytridiomycosis-infected cane toads (*Bufo marinus*), a recently introduced species, have been found (29), and in North America, bullfrogs (*Rana catesbeiana*) and other species of amphibians have been translocated or introduced widely. Some authors have suggested that tourists or fieldworkers surveying amphibian populations may have facilitated the dissemination of *Batrachochytrium* (19), although this has not been demonstrated. *Batrachochytrium* may have coevolved with some amphibians (e.g., lowland) species, populations of which remain unaffected. Recent disturbances of rain forest habitats may have introduced this parasite into naïve populations in Central America and Australia, leading to mass deaths. This range of disease outcomes parallels many diseases of humans, e.g., measles and smallpox, which produce a range of effects on persons in disease-endemic regions and cause massive deaths when introduced into naïve populations (48).

The occurrence of chytridiomycosis in free-living North American amphibians (Table 1) suggests a less obvious pattern of dissemination than in Central America and Australia. This irregularity may be due to a paucity of data, the pathogen's being enzootic to the United States, or the pathogen's introduction a number of years before. Historical reports of declines in the United States include postmetamorphic death syndrome, which progressed in waves through populations of amphibians, causing 90% to 100% death rates in recently metamorphosed animals and low death rates in larvae (50). Recent reports of chytridiomycosis-linked die-offs in *Bufo boreas* markedly resemble these previous die-offs. This observation and the finding that chytridiomycosis caused similar die-offs (of *B. canorus* and *R. pipiens*) in the 1970s (Table 1) (32), support the last of the above hypotheses. In the United States an amphibian pathogen (histologically very similar to *Batrachochytrium* but identified as *Basidiobolus ranarum*) has been described in wild Wyoming toads (*Bufo hemiophrys baxteri*) (51) and captive dwarf African clawed frogs (*Hymenochirus curtipes*) (52). As the latter species was widely introduced in ornamental garden ponds throughout the United States in the late 1980s, it may be involved in the dissemination of *Batrachochytrium*.

### Chytridiomycosis as the Cause of Population Declines

The ability of a pathogen to cause local population declines resulting in local host extinction requires a mechanism of persistence at low host densities. In epidemiologic models, highly virulent parasites rapidly suppress the host population density below a threshold value required to maintain transmission, resulting in the pathogen's extinction and recovery of the host population (7,10). Microparasites such as *Batrachochytrium*, with their relatively short duration of infection and high death rates, have an increased threshold population density and are usually less able to persist. Many parasites have evolved life history strategies for persistence (10) and the presence of reservoir hosts may augment the impact of other introduced wildlife diseases on host populations (46). The acinical presence of *Batrachochytrium* in the keratinized mouthparts of amphibian larvae implicates this life-cycle stage as a reservoir host for the pathogen. This form of infection may

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enable *Batrachochytrium* to persist in reduced amphibian populations (Figure 3). In both Australia and Central America, chytrid infection was observed in larval mouthparts months after initial adult deaths: the larvae of many tropical amphibian species survive 12 to 18 months—and some temperate species as long as 3 years—before metamorphosing. Examples of larval infection enhancing pathogen-mediated population declines and leading to host population extinctions have been reported for invertebrates (53).

Persistence may be further enhanced by saprophytic development (Figure 3). *Batrachochytrium* can be cultured in vitro on tryptone agar without the addition of keratin or its derivatives (37,42), and it will grow for at least one generation on cleaned epidermal keratin or on amphibians that have died of the infection (42). *Batrachochytrium* may survive and reproduce as a saprophytic organism in the environment, at least for short periods. Keratin (from decaying carcasses, shed skin, and other sources) is widely distributed in the environment, and chytrids that use this substrate are

well known (42). Furthermore, the ability to develop and reproduce saprophytically is common to many other fungal (54) (including chytrid [41]) and bacterial (55) pathogens. An epidemiologic model of a host-parasite system for pathogens that can reproduce saprophytically clearly shows a lowering of the host threshold population, allowing the pathogen to drive the host to extinction (55). Development of *Batrachochytrium* for even short periods outside its amphibian host may greatly increase its impact and accelerate population declines. Long-term presence as a saprophyte may explain the lack of recolonization of streams from which amphibians have been extirpated in both Australia (29) and Central America (24,25).

The impact of chytridiomycosis may be enhanced by the ecologic characteristics of certain host species. In Australia, chytridiomycosis-linked deaths have occurred in both declining and nondeclining species (28,29). Species with declining populations belong to a similar ecologic guild: regionally endemic rain forest specialists with low fecundity that

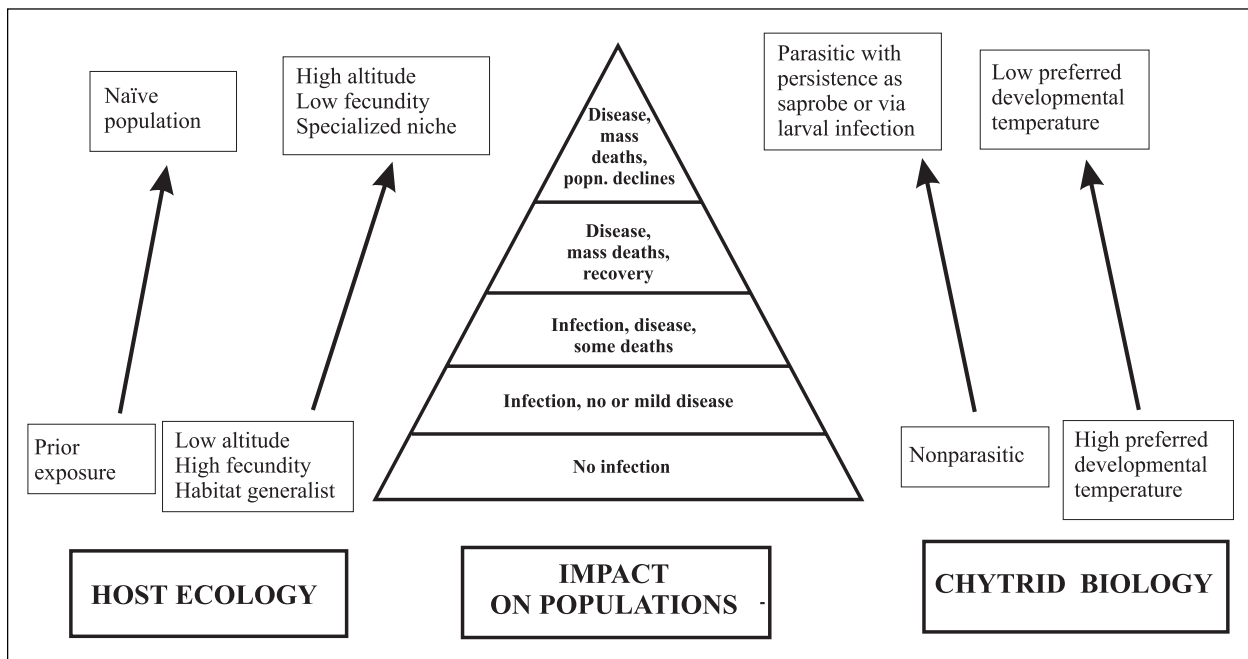


Figure 3. Diagrammatic representation of the range of disease outcomes in populations of amphibians affected by a *Batrachochytrium*-like pathogen. Factors that hypothetically predispose some amphibian populations to declines are illustrated. In this model, host ecologic traits (left side of pyramid) and parasite biologic traits (right side of pyramid) combine to produce declines in a specific group of amphibian species that have low fecundity, are stream-breeding habitat specialists, and occur in montane regions. These characteristics predispose them to population declines after introduction of a waterborne pathogen with a low preferred developmental temperature and ability to persist at low host population densities.<sup>1</sup>

<sup>1</sup>Note that the relative number of mass deaths decreases with increasing impact on population.

reproduce in streams and live at high altitudes (22). These characteristics, which are largely shared by declining Central American amphibians (24,25), are predictors of increased impact from chytridiomycosis. Species that reproduce in streams are probably more susceptible to a waterborne pathogen than terrestrial breeders. Low fecundity (56) and habitat specialization indicate a reduced ability to recover from population declines caused by stochastic events, including disease introduction. Laurance, McDonald, and Speare (39) suggested that the relation between high-altitude populations and declines may be due to a pathogen with a lower preferred developmental temperature. Preliminary data on cultured *Batrachochytrium* are consistent with this hypothesis: it develops most rapidly at 23°C in culture, with slower growth at 28°C and reversible cessation of growth at 29°C (42). The growth rate of *Batrachochytrium* in the skin (and therefore virulence) and the survival of zoospores outside the host (and therefore transmission rate) are likely to be lower in amphibians from the warmer lowland regions. The ability of the pathogen to survive saprophytically in the environment and for the disease to persist may also be enhanced in the cooler montane regions. These laboratory data may explain why chytridiomycosis has been associated with population declines in North American amphibians in montane localities (31,32) and after periods of cool weather at many U.S. and Australian sites (29,31).

### Potential Environmental Cofactors in the Emergence of Chytridiomycosis

Multiple factors (host, pathogen, environmental) may be involved in chytridiomycosis emergence. Some authors have hypothesized that infectious disease is only the proximate cause of declines and that environmental factors such as increased UV-B, chemical pollution, climate change, or stress may have predisposed amphibian populations to opportunistic pathogens (13,32,57). Recent work at Monteverde, Costa Rica, suggests that atmospheric warming, with a resultant elevation of the average altitude of the base of the orographic cloud bank and an increase in dry periods, is causally linked to amphibian declines at this site (57). Although no pathologic studies of amphibians were undertaken, overcrowding during periods of drought

may have allowed chytridiomycosis to cause substantial deaths (57). Further work is required to test this hypothesis, since a drier climate would also predict a lower overall impact from chytridiomycosis—a disease transmitted by flagellated, waterborne zoospores. The evidence suggests that cofactors are not required for chytridiomycosis to cause amphibian mass deaths. Chytridiomycosis is highly pathogenic to captive-bred amphibians exposed in captivity where control animals remained healthy (28,42). Further experimental infections using extremely small inocula (100 zoospores) also proved fatal (29).

Some deaths among wild amphibians have been attributed to immunosuppression, predisposing them to infectious disease (13,32). In the chytridiomycosis-related deaths, chytridiomycosis was consistently found as the cause of death, and the range of opportunistic infections expected to occur in immunocompromised animals was not found (28,29). An increase in UV-B irradiation may influence amphibian declines (58), but in the subtropical regions of Australia and Central America, data demonstrate no significant increase in UV irradiation (29,59). Even so, the potential effect of increased irradiation on montane riparian rain forest amphibians is uncertain, since these animals lay eggs under rocks or in sand banks and adults are rarely exposed to direct sunlight (24,29). Furthermore, in these regions, the species most likely to be affected by UV increases (arboreal amphibians, which bask or lay exposed eggs) are not in decline (20). Despite extensive research, chemical pollution (20,25), habitat destruction (22), or climate change (57) have not, so far, been causally linked either to the Australian declines or to those at Central American sites other than Monteverde. No other possible cofactors, such as sympatric pathogens, have been found.

### Emerging Viral Diseases of Amphibians

Iridoviruses have been implicated as the cause of amphibian mass deaths worldwide, with novel iridoviruses of amphibians recently identified from a number of regions (Tables 1, 2). The Iridoviridae encompass five recognized genera: *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystivirus*, and goldfish virus 1-like viruses (71). Of these, the genus *Ranavirus* contains pathogens of fish, amphibians, and reptiles (Table 2; Figure 4).

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### Characteristics of Ranaviral Disease

Ranaviruses are often highly virulent and cause systemic infections in amphibians. Experiments with Bohle iridovirus and Gutapo virus suggest that tadpoles are the most susceptible developmental stage for ranavirus infection, and death rates of 100% occur (72). Infected metamorphs die without overt signs of

infection, and infected adults show either no overt signs or, occasionally, a general weakness. Histologically, acute necrosis of hematopoietic and lymphoid tissues and of leukocytes occurs in most organs of infected animals (72). Epizootiologic data on tadpole edema virus infections of North American amphibians are scanty, although the virus was isolated mostly from diseased animals

Table 2. Iridoviruses<sup>a,b</sup> of herpetofauna (34)

Host	Virus <sup>c</sup>	Country or region where isolated	Ref.
Amphibian iridoviruses	Frog virus 3	North America, United States	60
Leopard frog ( <i>Rana pipiens</i> )	considered type for sympatric isolates frog virus 1, 2, 9-23		
Red-spotted new eft <i>Notophthalmus viridescens</i> )	T6-20	North America, United States	61
Bullfrog ( <i>Rana catesbeiana</i> )	Tadpole edema virus	North America, United States	62
Edible frog ( <i>Rana esculenta</i> )	Rana esculenta iridovirus	Europe (Croatia)	63
Ornate burrowing frog ( <i>Limnodynastes ornatus</i> )	Bohle iridovirus	Australia	64
Cane toad ( <i>Bufo marinus</i> )	Gutapo iridovirus	South America (Venezuela)	65
Common frog ( <i>Rana temporaria</i> )	Rana UK virus	Europe, United Kingdom	33
Common toad ( <i>Bufo bufo</i> )	Bufo UK virus	Europe, United Kingdom	34
Red-legged frog larvae ( <i>Rana aurora</i> )	Redwood Creek virus	California, United States	66,67
Tiger salamander ( <i>Ambystoma tigrinum stebbinsi</i> )	<i>A. tigrinum</i> virus	Arizona, United States	27
Tiger salamander ( <i>A. t. mavortium</i> )	Regina ranavirus	Saskatchewan, Canada	36
Ranid frog ( <i>Rana grylio</i> )	Rana grylio virus	China	68
Tiger salamander ( <i>A. tigrinum</i> )	Not yet named	N. Dakota, United States	35
Spotted salamander ( <i>A. maculatum</i> )	Not yet named	Maine, United States	35
Tiger salamander ( <i>A. tigrinum</i> )	Not yet named	Utah, United States	35
Reptile iridoviruses			
Box turtle ( <i>Terrapene c. carolina</i> )	Turtle virus 3	Maryland, United States	69
Central Asian tortoise ( <i>Testudo horsfieldi</i> )	Tortoise virus 5	North America, United States	69
Soft-shelled turtle ( <i>Trionyx sinensis</i> )		China	70
Green tree python ( <i>Chondropython viridis</i> )	Wamena virus	Australia	A.D. Hyatt (unpubl. obs.)
Gopher tortoise ( <i>Gopherus polyphemus</i> )		North America, United States	69

<sup>a</sup>Erythrocytic viruses, which are antigenically unrelated to ranaviruses and are not associated with amphibian mass deaths or declines, are not included. Further work is required to evaluate their significance.

<sup>b</sup>There is little variation in the major capsid protein (a major antigen of this group of viruses) within the genus *Ranavirus* (<4% difference at the nucleotide and amino acid level; Hyatt, unpubl. obs). This high degree of homology is interesting, as some of these viruses do not appear to be species specific. No discriminating neutralizing antibodies exist, and ranaviruses are identified and characterized by a range of techniques, including antigen capture enzyme-linked immunosorbent assay, polyacrylamide gel electrophoresis, restriction endonuclease digestion, polymerase chain reaction, and sequencing and in situ hybridization (67,71).

<sup>c</sup>Where no name has been given, the virus has not yet been named.



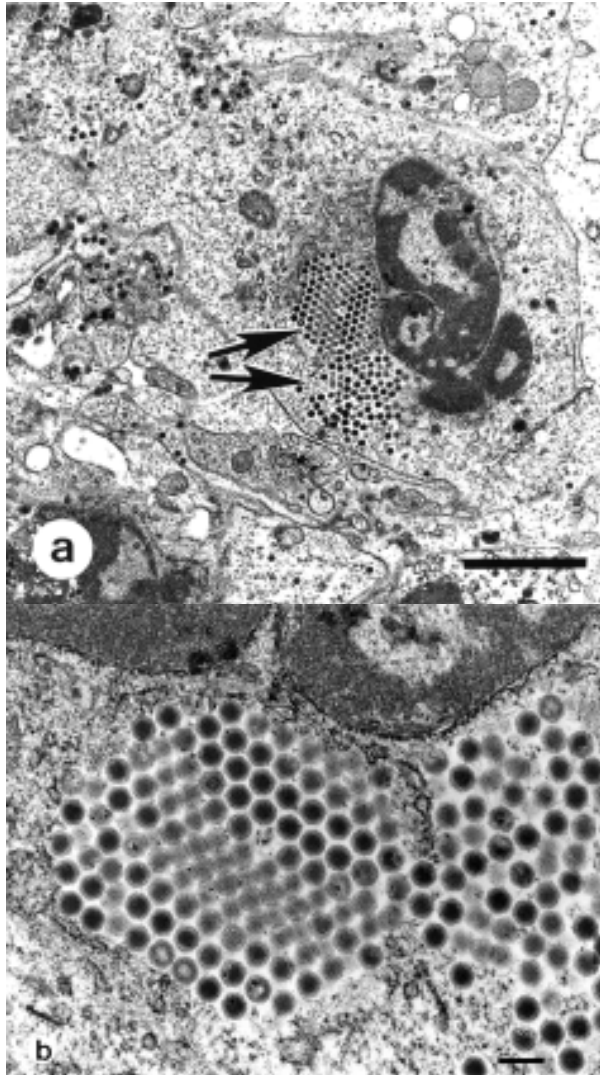


Figure 4. Transmission electron micrographs of iridovirus cultured from the liver of a naturally diseased common frog (*Rana temporaria*) by using a fathead minnow epithelial cell line. 4a. Virus-infected cell. Large isocahedral viruses are conspicuous within the cytoplasm (arrows). Bar = 2  $\mu$ m. 4b. Paracrystalline array of iridovirus. Bar = 200  $\mu$ m.

(60-62). Experimental infections show that bullfrog (*R. catesbeiana*) tadpoles infected with tadpole edema virus develop an acute lethal disease after a short incubation. The virus invades the liver, kidneys, and digestive tract and causes hemorrhage in skeletal tissue, pathologic findings similar to those described in *B. marinus* infected with the ranaviruses frog virus 3, Bohle iridovirus, or Gutapo virus (72). Tadpole edema virus was uniformly lethal to three species of experimentally infected North

American toad (61,62). Experimental inoculation with tadpole edema virus caused high rates of deaths in toads (100%, 4 to 17 days postinfection in young and adult *Bufo* sp. and newly metamorphosed *Scaphiopus* sp.), 40% death rates in metamorphosing bullfrogs (*R. catesbeiana*), and no deaths in newly hatched salamanders (*Ambystoma* sp.). For the last two species, the low virulence suggests either some prior exposure and acquired immunity or a degree of innate resistance.

Pathologic data from ranaviral infections in wild amphibians may be complicated by secondary bacterial infection. Cunningham et al. (26) described two syndromes in *Rana temporaria* collected at sites of mass deaths in the United Kingdom: a hemorrhagic syndrome affecting the skeletal musculature and the alimentary and reproductive tracts and an ulcerative skin syndrome with dermal ulceration and often necrosis of the distal limbs, but without hemorrhage in the muscles or viscera. In some areas, frogs were found with lesions common to both syndromes (26). These lesions are characteristic of red-leg, a syndrome thought to be caused by bacterial infection; however, Cunningham et al. (26) concluded that the lesions they described were caused by primary iridoviral infection, with or without secondary bacterial infection. Secondary bacterial infection was also reported in wild-collected, diseased *A. t. stebbinsi* infected with *Ambystoma tigrinum* virus (27). This infection resulted in rapid onset (5 to 7 days) of apparent epidermal hyperplasia, then dermal hemorrhage, followed by visceral hemorrhage and death rates of up to 45% (27).

### Factors Associated with the Emergence of Ranaviruses

The epizootiology of ranaviral disease in amphibians is poorly understood. Data from closely related ranaviral infections of fish, however, suggest a number of factors which may explain their recent emergence. Epizootic hematopoietic necrosis virus is a ranaviral pathogen of fish and the causative agent of epizootic hematopoietic necrosis, a notifiable disease under the Office Internationale des Epizooties (73,74). Sequencing data suggest that this disease is unique among the ranaviruses, is probably indigenous to Australia, and has a wide geographic range. Dissemination may be partly due to the virus's ability to remain infectious

under adverse conditions and for prolonged periods (Table 3).

The resistant nature of epizootic hematopoietic necrosis virus suggests that amphibian iridoviruses may survive long periods at the bottom of ponds, particularly during winter. Jancovich et al. (27) demonstrated that water containing *Ambystoma tigrinum* virus-infected *Ambystoma* became uninfected after 2 weeks at 25°C, and epizootics of *Ambystoma tigrinum* virus and regina ranavirus often followed periods of cold weather (27,36). Epizootic hematopoietic necrosis virus may be spread by fomites such as fishing nets, boats, and fishing rods and through artificial stocking of ponds for recreational fishing (76). Birds have the potential to mechanically transfer virus on their feathers, feet, or bills, or by regurgitation of ingested infected material (76). Similar modes of spread are likely to occur with the amphibian ranaviruses. The occurrence of epizootic hematopoietic necrosis may be correlated with poor husbandry, including overcrowding, inadequate water flow, and fouling of local environments with feed from fish farms (77).

Recent movements of amphibians may have disseminated ranaviral diseases. The North American bullfrog (*Rana catesbeiana*), the host of tadpole edema virus, has been widely introduced in the western United States and South America. In the United Kingdom, ranaviruses may be disseminated by the common practice of translocation of amphibian egg masses and larvae by humans. Zupanovic et al. (65,78) reported the existence of ranaviruses and antibodies to ranaviruses in the cane toad (*Bufo*

*marinus*) in Venezuela. In Australia, where *B. marinus* was introduced to Queensland in 1935, ranaviral antibodies can be identified in this species throughout its range (78). The range of *B. marinus* has expanded rapidly in Australia, and the toad threatens environmentally sensitive wetlands in the Kakadu National Park. The effects of toxicity, predation, and competition of this relentlessly expanding species are well documented, and its potential to disseminate novel viral pathogens to native amphibians should also be considered in evaluating its ecologic impact. So far, a ranavirus (Bohle iridovirus) has been isolated from only one native Australian amphibian, the ornate burrowing frog (*Limnodynastes ornatus*) (79).

Experimental infection with Bohle iridovirus causes illness and death in a range of frogs and toads, fish, and reptiles (L. Owens, pers. comm.) (80,81). Gutapo virus, isolated from Venezuelan *B. marinus* (65), is also able to infect other amphibian species (72). Despite Bohle iridovirus' low host specificity, current levels of surveillance have not implicated it in diseases of free-ranging Australian animals other than those from which it was originally isolated. The ability of Bohle iridovirus to infect fish implicates the widespread introduction of fish for recreational purposes as a potentially significant factor in the dissemination of amphibian ranaviruses. Similarly, Redwood Creek virus, a ranavirus found to cause death of the endangered red-legged frog (67), can infect both amphibians and fish.

### Impact of Ranaviral Disease on Amphibian Populations

The link between amphibian population declines and ranaviral disease is less clear than that with chytridiomycosis. However, these highly virulent ranaviruses are a potential threat to amphibian populations, especially populations isolated from previous disease outbreaks (and thus lacking specific immunity) and species with low fecundity. In particular, the high death rates of tadpoles infected by ranaviruses predict a negative impact on populations. Using the model of Lampo and De Leo (82), a tadpole death rate of 80% to 90% would remove 80% to 90% of the animals that would otherwise survive metamorphosis, resulting in an approximately 80% reduction in adult populations (Lampo, pers. comm.). Although such an epizootic might not endanger the long-

Table 3. Longevity of infectious epizootic hematopoietic necrosis virus (72,74,75)

Treatment	Survival time (days)	Ref.
Animal tissues		
-20°C	>730	75
-70°C	>730	75
In solution		
Distilled water (temperature not specified)	97	75
4°C, cell maintenance media	>1124	74
-20°C, cell maintenance media	>1124	74
-70°C, cell maintenance media	>1124	74
On dry surfaces	>113, <200	75

term survival of a highly adaptable, highly fecund species such as *B. marinus*, species that inhabit specialized ecologic niches or have low fecundity might be adversely affected.

Recent outbreaks of iridoviral disease have been particularly notable in *Rana temporaria* (United Kingdom) and *Ambystoma tigrinum* (Arizona, Saskatchewan). These outbreaks involved extensive deaths and the annual recurrence of the diseases. Common ecologic themes occur for these amphibian populations. The natural habitat of *A. t. stebbinsi* has been entirely destroyed by drainage after human colonization of the southwestern United States, and relict populations are now confined to manmade watering holes for cattle. Other outbreaks of ranaviral disease in *A. tigrinum* have been associated with altered habitats and artificial ponds. Similar habitat destruction has occurred in the United Kingdom, where most outbreaks of iridovirus infection are reported from artificial ponds (16). High population densities of frogs (16) and salamanders (27) occur in these bodies of water, facilitating the transmission of viruses and predisposing the amphibian populations to mass deaths.

### Implications of Emerging Infectious Diseases for Amphibian Population Declines and Wildlife Conservation

The geographic spread of chytridiomycosis threatens populations of endemic and endangered amphibians in rain forests of Australia and Central and South America. Chytridiomycosis has now been reported from 38 amphibian species in 12 families, including ranid and hylid frogs, bufonid toads, and plethodontid salamanders (28,32,37,38,42,83). Although many of these records are from captive animals, the data demonstrate that chytridiomycosis is fatal to species originating from Europe, Africa, Madagascar, the Americas, and Oceania and attest to its potential impact should the disease be introduced into these areas. The emergence of amphibian ranaviruses raises similar concerns. Their ability to infect a wide range of amphibian and fish hosts (different vertebrate classes), global distribution, and high virulence clearly establish them as a global threat to amphibian populations. Despite the recent geographic spread and impact of chytrids and ranaviruses, these diseases probably do not account for all the reports of declining amphibian populations, and

evidence exists for other causal factors, such as habitat loss (12). Although chytridiomycosis causes high death rates in a range of species and habitats, it has caused population declines of amphibians only in certain species confined to montane rain forests, while the emergence of ranaviral disease seems to be associated with disturbed or degraded habitats.

Hypotheses regarding the origins and impact of emerging infectious diseases on amphibians are being tested. A number of *Batrachochytrium* and iridovirus isolates have been cultured, and analysis of their phylogenetic and pathologic features in natural and experimental infections, as well as their biologic characteristics (e.g., ability to survive in the environment, evolution of virulent strains) has already begun. Basic host and parasite ecologic data, such as prevalence and duration of infection and presence of reservoir hosts, are being collected. Data from these studies may enable the formulation of management plans to limit the diseases' impact. Development of chemotherapeutic regimens and diagnostic tests (enzyme-linked immunosorbent assay, in situ hybridization) may enable rapid progress toward these goals (29). National and international structures for the rapid dissemination of information between scientists, politicians, and the public may be crucial in combating the threat of these globally emergent pathogens. However, large geographic areas (e.g., Africa and much of Asia) have not yet been surveyed for declining amphibian populations or for the occurrence of these pathogens. Raising awareness of this threat should be one of the highest priorities for the immediate future.

Many introduced pathogens have affected human populations (10,47). However, far fewer studies of introduced diseases among wildlife have been published, and usually only those producing obvious population losses are studied vigorously (1-6).

The loss of amphibian populations to the point of local extinction is a striking effect of chytridiomycosis in Australia and Central America. However, the effects of introduced wildlife diseases may be more far-reaching and subtle, with knock-on (ripple) effects permeating throughout the ecosystem (1,2,7,82,84). In many tropical and some temperate areas, amphibians make up a significant proportion—sometimes most—of these vertebrate biomass (85), and their loss is likely to have unpredictable effects on

populations of other species. For example, loss of herbivorous amphibian larvae may cause an overgrowth of algae in montane tropical streams, with further knock-on effects. In Australia and Central America, certain species of snakes prey exclusively on amphibians and are likely to suffer significant population declines, in the same way that the red fox population was drastically reduced in the United Kingdom after the introduction of myxomatosis (10). Such insidious effects following disease introduction and an underestimation of their historical incidence suggest that this pathogen pollution may be as serious a conservation threat as habitat destruction and chemical pollution (1).

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