AMPHIBIAN DECLINE:

An Integrated Analysis of Multiple Stressor Effects

Greg Linder, Sherry K. Krest, Donald W. Sparling

A Publication of SETAC®

Biotic Factors in Amphibian Population Declines

Cynthia Carey, David F. Bradford, Jesse L. Brunner, James P. Collins, Elizabeth W. Davidson, Joyce E. Longcore, Martin Ouellet, Allan P. Pessier, Danna M. Schock

Introduction to Biotic Stressors

Amphibians have evolved and continue to exist in habitats that are replete with many other organisms. Some of these organisms serve as prey for amphibians, while others interact with amphibians as predators, competitors, pathogens, or symbionts. Still other organisms have no observable relationship with amphibians. As range expansions and emergence of new species have brought amphibians into contact with new organisms, novel relationships that have proven detrimental, neutral, or beneficial to amphibians have become established. This process has occurred naturally over time, independent of human involvement. Success of amphibians during their 350 million–year existence must have depended in part on their ability to deal effectively with contact with new species. Recently, however, amphibians have been presented with new challenges by human transport of novel pathogens, parasites, predators, or competitors into amphibian habitats. Furthermore, human modifications of the environment may have fostered the emergence of new pathogens and/or diminished the ability of amphibians to mount immune defenses against pathogens.

Pathogens capable of causing amphibian mortalities have certainly existed since amphibians evolved. Ecologists increasingly acknowledge the role of pathogens in population dynamics (Real 1996) and in maintaining diverse communities and ecosystems (McCallum and Dobson 1995). Recent mass mortalities described in this chapter are unusual because of the large number of species affected by pathogens, the rapidity with which host populations have declined, and the geographic scope of these mortalities. The complexity typical of pathogen–vector–host interactions serves as a challenge, however, for biologists trying to understand the role of disease in ecosystem processes from individual through population to landscape levels of organization. Mathematical models will be used with greater frequency in the future to help untangle this complexity by specifying conditions that allow infected populations to persist and by clearly specifying the various population parameters that must be estimated. The complexity of ecological systems makes testing and refining these models difficult because boundaries of host and vector populations are often ill-defined, several vectors can interact among themselves and with the host, and environmental conditions may alter interactions. In addition, except for certain disease processes in humans, few data exist for testing predictions of mathematical mod-

Amphibian Decline: An Integrated Analysis of Multiple Stressor Effects. Greg Linder et al., editors.
© 2003 Society of Environmental Toxicology and Chemistry (SETAC). ISBN 1-880611-55-4

els (Grenfell and Harwood 1997). For wildlife, we simply do not know enough yet about the details of the causes of disease outbreaks, how pathogens move among habitats, how pathogens affect host and vector life history, and how spatial arrangement or quality of host habitats affect disease persistence in animal populations (Anderson and May 1991; Lloyd and May 1996; Real and McElhany 1996; Frank 1997; Grenfell and Harwood 1997; Hanski 1997).

This chapter will examine the evidence concerning how three aspects of the biotic environment (pathogens, invasive species, and biotic factors implicated in the development of limb deformities) might serve as direct causes of amphibian population declines and extinctions. The ability to provide convincing demonstrations of causality has been complicated by a general lack of information about the natural fluctuations in population sizes and possible influences of one or more aspects of the physical environment. However, demonstration of a direct causal relationship between pathogens and amphibian declines is probably less problematic than for other factors, such as ultraviolet light or contaminants, because fulfillment of Koch's postulates promotes idenfication of specific pathogen-causing infectious disease (see Chapters 3 and 8A). These procedures include isolation and purification of the potential pathogen, exposure of uninfected animals to this pathogen, development of the disease with the same signs as noted in the originally infected animals, and then re-isolation and purification. These postulates must be met before a pathogen can be determined to be the direct cause of death. An example of the procedures for verification of Koch's postulates is provided by Nichols et al. (2001). Although a number of studies implicate pathogens in amphibian mass mortalities in the field (cf. Hird et al. 1981; Worthylake and Hovingh 1989; Bradford 1991; Carey 1993), identification of the causal pathogen is not possible without the performance of complete necropsies.

Invasive species and parasite-induced deformities offer new challenges in determining cause-and-effect relationships with amphibian declines because tools for evaluating potential relationships are in their infancy. However, as noted in this chapter, attention has begun to focus beyond simple correlations to establishment of criteria by which cause and effect can be demonstrated. This chapter should stimulate research in these directions.

Amphibian pathogens

Although a wide variety of pathogens and infectious diseases has been documented in amphibians (Crawshaw 2000; Taylor 2001; Taylor et al. 2001), convincing documentation of a causal relation between pathogens and mass mortalities and declines in population sizes of amphibians exists for only two types of pathogens. The first is a chytrid fungus, *Batrachochytrium dendrobatidis,* that causes chytridiomycosis, an infection of amphibian skin. Population declines, extirpation of local populations, and possibly even species extinctions on at least four continents and Central America have been attributed to this pathogen (Berger et al. 1998; Ron and Merino 2000; Bosch et al. 2001; Muths et al. 2003; see review by Carey 2000). The second class of pathogen, a group of iridoviruses of the genus *Ranavirus,* is associated with mass mortalities of amphibians but has not yet been proven to cause permanent population declines.

Chytridiomycosis

Although the causes of many amphibian declines, such as habitat destruction, are obvious, a large number of mysterious deaths or disappearances of amphibians have occurred over the last 30 years in areas that are relatively free of human impacts (see review by Carey 2000). Several of these declines were attributed to disease, but incomplete necropsies may have prevented accurate identification of the pathogen (Bradford 1991; Carey 1993). In 1998, Berger et al. implicated a chytrid fungal disease as a cause of widespread declines of amphibian populations in protected montane areas of Australia and Central America. They were able to transmit this disease by exposing uninfected *Mixophyes fasciolatus* frogs to skin scrapings from a frog of the same species that had recently died. Infection and death, or euthanasia in cases which animals were moribund, followed within 10 to 18 days (Berger et al. 1998). Zoo personnel at the Smithsonian's National Zoological Park in the Unites States had been successfully raising poison dart frogs (*Dendrobates spp.*) for years when suddenly a number of metamorphosed young succumbed to infectious disease (Pessier et al. 1999). A chytrid fungus, which was histologically the same as that reported by Berger et al. (1998), was associated with these deaths. Veterinary pathologists from the National Zoo and a mycologist from the University of Maine established a pure culture of the chytrid (Pessier et al. 1999), named it as a new genus and species (Longcore et al. 1999), and used the pure culture to show that this chytrid, by itself, was capable of causing infection and death of metamorphosed poison dart frogs. Koch's postulates have been fulfilled for this disease organism by reproducing the disease and isolating the same fungus from animals that died after being inoculated with a pure culture of the suspected agent (Nichols et al. 2001).

What is a chytrid?

"Chytrid" is the common name for members of the Chytridiomycota, the phylum of the earliest diverging lineage of fungi. Other phyla of fungi include the Zygomycota, the Ascomycota, and the Basidiomycota (Alexopoulos et al. 1996). Unlike these phyla, most members of the Chytridiomycota produce reproductive spores that swim by means of a single posteriorly directed flagellum. These motile reproductive bodies are surrounded by a cell membrane, and they lack the chitinous wall possessed by the vegetative stage. Thus, with only a membrane to protect the cell contents, spread of chytrid spores requires free water.

The Chytridiomycota contains 123 genera in 5 orders, with the amphibian chytrid being a member of the largest but most inadequately classified order, the Chytridiales (Berger et al. 1998; Longcore et al. 1999; Pessier et al. 1999). The diversity of species in this group is largely unknown, and discovery of new species and genera is still common (Powell 1993). The phylogenetic relationships of taxa within the Chytridiales are just now beginning to be identified with ultrastructural (Barr 1980, 2000) and molecular (James et al. 2000) characters. Chytrids are common in soil and water. They inhabit organic substrates (e.g., keratin, chitin, pollen, cellulose, leaves) as well as being saprobic or parasitic on or in algae, protozoa, and other fungi. Some species are specific to a particular substrate, whereas other species are generalists.

When thinking of fungi, many people visualize a thread-like mycelium, but many chytrids, and particularly many members of the Chytridiales, are not mycelial. Instead the body, or thallus, of the fungus has determinate growth. The motile, asexual, reproductive spore (zoospore) can withdraw its flagellum, settle down on a substrate, and form a wall (encyst). This encysted zoospore may develop directly into a zoospore-producing structure called a "sporangium," or the cyst may produce a germ tube that enters the substrate and the sporangium may be formed from a swelling in the germ tube within the substrate. Rhizoids (root-like processes) anchor the developing sporangium and absorb nutrients. When sporangia are mature, the cytoplasmic contents divide into uninucleate zoospores. Motile zoospores are released through discharge pores or papillae.

Population declines associated with chytridiomycosis

Because amphibians naturally undergo fluctuations in population sizes, it can be difficult to prove that declines have been caused by a specific pathogen. Several case studies stand out, however, because 1) populations were monitored prior to a mass mortality event so that the decline in population size was obvious; 2) sick and/or freshly dead animals were available for complete postmortem examinations that not only verified this fungus as the direct cause of death but also ruled out other possible causes of death; and 3) most or all the animals in the population died. These well-documented declines, proven to result from chytridiomycosis, occurred at Big Tableland, Queensland, Australia (Berger et al. 1998); Fortuna Forest Reserve, Panama (Berger et al. 1998); Pen˙alara Natural Park, Spain (Bosch et al. 2001); and Rocky Mountain National Park, Colorado, U.S.A. (Muths et al. 2003). Several historical die-offs of amphibians in the 1970s are also thought to have been caused by this pathogen because histological analyses of museum samples collected at the times of the die-offs revealed the presence of this pathogen (Carey et al. 1999; Green and Sherman 2001). Animals infected with *B. dendrobatidis* have also been found at some sites at which population declines have not been documented (Maine [J.R. Longcore, United States Geological Survey (USGS), Orono, Maryland, USA, personal communication], Quebec [M. Ouellet, Redpath Museum, McGill University, Montreal, Quebec, Canada, personal communication] and British Columbia [Raverty and Reynolds 2001]). The presence of these chytrid fungi in amphibian populations that are not known to be declining could be explained by 1) mass declines due to this pathogen might have occurred years ago, and surviving animals may be resistant to lethal infection by this pathogen; 2) population surveys and baseline data on population sizes are inadequate to detect declines; or 3) the ability of the fungus to cause lethal infections may be influenced by local environmental conditions and/or species variation in susceptibility.

Incidences of chytridiomycosis

The chytrid fungus *B. dendrobatidis* has been identified on a wide variety of both anuran and caudate amphibians, including members of the Ambystomatidae, Amphiumidae, Bufonidae, Centrolenidae, Dendrobatidae, Discoglossidae, Hylidae, Ranidae, Leptodactylidae, Microhylidae, Pipidae, Proteidae, and Sirenidae (Table 6-1). Infections have been found on all continents on which amphibians exist except for Asia (Speare and Berger 2000a, 2000b), and new sites are continuously being added (Burrowes and Joglar 2002; NWHC 2003; J.R. Longcore, USGS, Orono, Maryland, USA, personal communi-

cation). The lack of data from Asia more likely reflects the lack of samples for examination than the absence of this pathogen. This chytrid has been identified on amphibians in a number of U.S. states, several Canadian provinces, Central America, both eastern and western Australia, a limited area of New Zealand, and in several locations in Europe and Africa (Table 6-1). Isolates of *B. dendrobatidis* do not appear to be specialized to a single species, family, or order; an isolate derived from a caudate has been shown in the laboratory to infect an anuran, and vice-versa (Davidson et al. 2000). Boreal toads, *Bufo boreas*, vary significantly in time to death after exposure to various strains of *Batrachochytrium* isolated from several species of amphibians, including Panamanian anurans, *Ambystoma tigrinum,* and *Pseudacris triseriata* (L.J. Livo et al., University of Colorado, Boulder, Colorado, USA, unpublished data).

Table 6-1 Geographic origins and hosts of chytridiomycosis (also see Speare and Berger 2000a, 2000b)

Area	Host Species	Comments	Citation
Africa			
Ghana	*Xenopus (Silurana) tropicalis* [a]	Imported to U.S.A.	J.E. Longcore, University of Maine, Orono, ME, USA, unpublished data
Western Africa	*Xenopus (Silurana) tropicalis* [a]	Imported to U.S.A.	Reed et al. 2000
Kenya	*Xenopus (Silurana) tropicalis* [a]		Speare and Berger 2000b
South Africa	*Ptychadena anchietae*		Speare and Berger 2000b
	Xenopus laevis		Speare and Berger 2000b
Australia			
Queensland	*Litoria* (10 spp.)		Speare and Berger 2000a
	Nyctimystes dayi		Speare and Berger 2000a
	Uperoleia laevigata		Speare and Berger 2000a
	Adelotus brevis		Speare and Berger 2000a
	Lechriodus fletcheri		Speare and Berger 2000a

[a] Species in captivity

continued

Table 6-1 continued

Area	Host Species	Comments	Citation
Queensland (continued)	*Limnodynastes* (2 spp.)		Speare and Berger 2000a
	Myxophyes (2 spp.)		Speare and Berger 2000a
	Taudactylus (2 spp.)		Speare and Berger 2000a
	Bufo marinus		Speare and Berger 2000a
	Ambystoma mexicanum[a]		Speare and Berger 2000a
Western Australia	*Litoria* (2 spp)		Speare and Berger 2000a
	Crinia (4 spp.)		Speare and Berger 2000a
	Geocrinia (2 spp.)		Speare and Berger 2000a
	Heleioporus eyrei		Speare and Berger 2000a
	Limnodynastes dorsalis		Speare and Berger 2000a
	Neobatrachus pelobatoides		Speare and Berger 2000a
	Ambystoma mexicanum[a]		Speare and Berger 2000a
South Australia	*Litoria* (2 spp.)		Speare and Berger 2000a
	Limndynastes (2 spp.)		Speare and Berger 2000a
Victoria	*Litoria* (2 spp.)		Speare and Berger 2000a
	Limnodynastes dumerilii [a]		Speare and Berger 2000a
	Mixophyes fasciolatus		Speare and Berger 2000a
	Neobatrachus kunapalari		Speare and Berger 2000a
	Pseudophryne pengileyi		Speare and Berger 2000a
	Taudactylus acutirostirs		Speare and Berger 2000a

[a] Species in captivity

continued

Table 6-1 continued

Area	Host Species	Comments	Citation
Victoria (continued)	*Bufo marinus* [a]		Speare and Berger 2000a
New South Wales	*Litoria* (7 spp.)		Speare and Berger 2000a
	Adelotus brevis		Speare and Berger 2000a
	Heleioporus australiacus		Speare and Berger 2000a
	Mixophyes (3 spp.)		Speare and Berger 2000a
	Pseudophryne (2 spp.)		Speare and Berger 2000a
Canada			
British Columbia	*Bufo boreas*		Raverty and Reynolds 2001
	Hymenochirus boettgeri [a]		Raverty and Reynolds 2001
Quebec	*Rana catesbeiana*	Not endangered	M. Ouellet, Redpath Museum, McGill University, Montreal, Quebec, CA, unpublished data
	Pseudacris triseriata	Vulnerable species	Desroches & Ouellet 2001
Costa Rica	*Atelopus varius*		Speare and Berger 2000b
Ecuador	*Atelopus* sp.		Ron and Merino 2000
	Atelopus bomolochos		Ron and Merino 2000
	Gastrotheca pseustes		Ron and Merino 2000
	Telmatobius niger		Ron and Merino 2000
Germany	*Dendrobates*[a] (3 spp.)		Mutschmann et al. 2000
	Phyllobates[a] (4 spp.)		Mutschmann et al. 2000
	Other captive species		Mutschmann et al. 2000

[a] Species in captivity

continued

Table 6-1 continued

Area	Host Species	Comments	Citation
Germany (continued)	*Rana arvalis*		Speare and Berger 2000b
New Zealand	*Litoria raniformis*	Introduced species from Australia	Waldman et al. 2001
	Leiopelma archeyi	Threatened species	Froglog 2001
Panama	*Atelopus chiriquiensis*		Berger et al. 1998
	Atelopus varius		Berger et al. 1998
	Bufo haematiticus		Berger et al. 1998
	Cochranella prosblepon		Berger et al. 1998
	Cochranella albomaculata		Berger et al. 1998
	Ileutherodactylus emcelae		Berger et al. 1998
	Eleutherodactylus cruentus		Berger et al. 1998
Spain	*Alytes obstetricans*	Mass mortalities in alpine park; steep population decline	Bosch et al. 2001
Uruguay	*Rana catesbeiana* [a]	Introduced; commercial production	Mazzoni 2000
U.S.A.			
Zoos	*Bolitoglossa dofleini* [a]		Nichols et al. 1998
	Bufo viridis[a]		Nichols et al. 1998
	Ceratobatrachus guentheri [a]		Nichols et al. 1998
	Ceratophris ornata [a]		Nichols et al. 1998
	Dendrobates auratus [a]		Nichols et al. 1998

[a] Species in captivity

continued

Table 6-1 continued

Area	Host Species	Comments	Citation
Zoos (continued)	*Dendrobates azureus* [a]		Nichols et al. 1998
	Dyscophagus guineti [a]		Nichols et al. 1998
	Litoria caerulea [a]		Nichols et al. 1998
	Mantella cowanii [a]		Nichols et al. 1998
	Bufo americanus [a]		J.E. Longcore, University of Maine, Orono, ME, USA, unpublished data
	Bufo woodhousei [a]		J.E. Longcore, University of Maine, Orono, ME, USA, unpublished data
Arizona	*Ambystoma tigrinum*		E.W. Davidson, Arizona State University, Tempe, AZ, USA, unpublished data
	Pseudacris triseriata		Miera et al. 2002
	Hyla arenicolor		Bradley et al. 2002
	Rana chiricahuensis	Proposed federally threatened	Bradley et al. 2002
	Rana yavapaiensis	State candidate for threatened species	Bradley et al. 2002
	Rana berlandieri		Sredl and Caldwell 2000
	R. blairi		Sredl and Caldwell 2000
California	*Bufo microscaphus californicus*	Fungus identified as "fungal-like protists"	Nichols et al. 1996
	Bufo canoris	Rapid population decline	Green and Sherman 2001
	Rana. muscosa	Tadpole mouthparts infected	Fellers et al. 2001

[a] Species in captivity

continued

Table 6-1 continued

Area	Host Species	Comments	Citation
California (continued)	*Hymenochirus curtipes*[a]	fungus misidentified as *Basidiobolus ranarum*	Groff et al. 1991
	Ambystoma marcodactylum croceum		NWHC 2003
	Hyla regilla		NWHC 2003
	Rana aurora draytoni	Threatened	Green et al. 2002
Colorado	*Bufo boreas*	State endangered; collected in 1974; museum samples	Carey et al. 1999 Muths et al. 2003
	Rana pipiens [a]	Collected in 1974; museum samples	Muths et al. 2003
Illinois	Acris crepitans		Nichols et al. 1996
Maine	*Rana pipiens*	Species of special concern.	J.R. Longcore et al., USGS, Orono, MD, USA unpublished data
	Rana sylvatica		J.R. Longcore et al., USGS, Orono, MD, USA, unpublished data
	Rana catesbeiana		J.R. Longcore et al., USGS, Orono, MD, USA, unpublished data
	Rana clamitans		J.R. Longcore et al., USGS, Orono, MD, USA, unpublished data
	Rana septentrionalis		J.R. Longcore et al., USGS, Orono, MD, USA, unpublished data
	Rana palustris		J.R. Longcore et al., USGS, Orono, MD, USA, unpublished data

continued

[a] Species in captivity

Table 6-1 continued

Area	Host Species	Comments	Citation
Maine (continued)	*Bufo americanus*		J.R. Longcore et al., USGS, Orono, MD, USA, unpublished data
Maryland	*Bufo americanus*		Milius 1998
Massachusetts	*Rana clamitans*		J.R. Longcore et al., USGS, Orono, MD, USA, unpublished data; NWHC 2003
New Mexico	*Rana chiricahuensis*	State protected group I	New Mexico Game and Fish Department
North Carolina	*Hyla gratiosa*		NWHC 2003
	Rana sphenocephala		NWHC 2003
	Rana catesbeiana		NWHC 2003
	Ambystoma maculatum		NWHC 2003
North Dakota	*Rana pipiens*		Green et al. 2002
Puerto Rico	*Eleutherodactylus karlschmidti*	Extinct; museum specimen	Burrowes and Joglar 2002
	Eleutherodactylus coqui	Museum specimens	Burrowes and Joglar 2002
South Carolina	*Rana catesbeiana*	Museum specimen	P. Daszak et al., Consortium for Conservation Medicine, Palisades, NY, USA, unpublished data
	Rana utricularia	Museum specimen	P. Daszak et al., Consortium for Conservation Medicine, Palisades, NY, USA, unpublished data
Utah	*Bufo boreas*	Species of special concern	NWHC 2003

continued

Table 6-1 continued

Area	Host Species	Comments	Citation
Utah (continued)	*Rana pretiosa*		NWHC 2003
Wyoming	*Bufo baxteri*	Endangered	NWHC 2003

Possible environmental cofactors

Because exposure to *B. dendrobatidis* readily kills susceptible amphibians in the laboratory in the absence of an environmental stressor, some researchers have concluded that environmental co-factors, such as ultraviolet-B (UV-B) or xenobiotics, are not necessary for this chytrid to cause widespread amphibian declines (Daszak et al. 1999). A large-scale analyses of weather patterns in four locations just prior to and during amphibian die-offs thought to have been caused by *Batrachochytrium* has recently been made by Alexander and Eischeid (2001). They found no consistent weather patterns at the four sites that might implicate temperature or moisture patterns as indirect causes of mass mortalities due to chytridiomycosis. Although significant increases in ultraviolet B light have occurred at many localities in Central and South American sites of amphibian declines, no evidence yet exists that UV-B exposures of amphibians, which are largely active in shade or at night, have increased coincidentally (Middleton et al. 2001). Although anecdotal observations exist that lethal chytridiomycosis occurs frequently at cold temperatures (for review see Carey 2000), mortality rates of amphibians exposed to chytrid zoospores at various temperatures are currently under examination.

Clinical signs and gross pathology

Both tadpoles and postmetamorphic animals can be infected, but morbidity and mortality have been demonstrated to date only in postmetamorphic (including adult) animals. Among postmetamorphic animals, both clinically apparent (causing illness or death) and clinically inapparent (subclinical) infections may be observed. Whether infections become clinically apparent or remain subclinical is largely undetermined, but may include a number of factors, including variation in individual species susceptibility to infection.

Compared to die-offs associated with other pathogens, such as iridoviruses, mortality events and declines due to chytridiomycosis usually appear slowly with small numbers of carcasses present at an affected site at any one time although cumulative numbers may be high (Green 2001). Individual manifestations of *Batrachochytrium* infection can be varied and many affected animals will have no externally visible evidence of disease other than being found moribund or dead. Of those with grossly visible lesions, changes in the skin are most common and consist of excessive shedding (sloughing) or discoloration of the skin usually of the ventral body and toes and feet (Pessier et al. 1999; Berger et al. 2000; Green 2001; Nichols et al. 2001). Discoloration is usually brown; however, in some species reddening (erythema), which can mimic other amphibian diseases such as the red-

leg syndrome, may be observed (Pessier et al. 1999; Taylor, Williams, and Mills 1999; Taylor Williams, Thorne, et al. 1999; Berger et al. 2000). In captivity or in wild animals found alive, lethargy and anorexia (lack of appetite) have been described in a variety of species (Nichols et al. 1996, 2001; Berger et al. 2000). Other behavioral abnormalities have included sitting unprotected during the day (Berger et al. 2000), abnormal resting postures (Berger et al. 2000; A. Pessier, University of Illinois, Maywood, Illinois, USA, personal observation), and avoidance of water (C. Carey et al., University of Colorado, Boulder, Colorado, USA, unpublished data). Neurologic signs, described from some Australian rainforest frogs, include rigidity and trembling and extension of the hindlimbs with flexion of the forelimbs (Berger et al. 2000). Manifestations of chytridiomycosis in tadpoles are largely limited to depigmented and deformed mouthparts (toothrows and beaks); thickening of the toe tips may be detectable in late-stage anurans and larval caudates (Fellers et al. 2001; Green 2001). No evidence of illness or mortality has yet been observed in tadpoles. Infected tadpoles may carry the pathogen through metamorphosis (Lamirande and Nichols 2002), but the effect of pre-metamorphic infection on size at metamorphosis and post-metamorphic survivability has not yet been determined.

Microscopic pathology

The microscopic pathology of chytridiomycosis is similar in all species and consists of variable epidermal hyperplasia with moderate to severe hyperkeratosis, which is a thickening of the outermost layers of the epidermis (stratum corneum). Within the thickened epidermal layers are numerous fungal thalli, which are simple spherical bodies with thread-like absorbtive structures (rhizoids). The thallus of a single organism visible with routine hematoxylin and eosin staining develops within the cytoplasm of a single skin cell (keratinocyte). In heavy infections (usually those that are clinically significant), thalli are distributed throughout the epidermis, whereas in early or low-level infections, fungal thalli generally occur as widely separated clusters. The characteristic features of individual thalli (Figures 6-1 and 6-2) that aid in identification of *B. dendrobatidis* include flask-shaped thalli, occasional round thalli containing discrete basophilic spores, and colonial thalli characterized by internal septation. The presence of colonial thalli or demonstration of rhizoids using a silver (Gomori methenamine silver [GMS]) stain can be used to diagnose infection consisting primarily of empty thalli or to distinguish chytrid infections from those caused by *Basidiobolus*. A review of original histological materials (A. Pessier, University of Illinois, Maywood, Illinois, USA, personal observation) revealed that fungal infections of dwarf African clawed frogs *(Hymenochirus curtipes)* (Groff et al. 1991), Wyoming *(Bufo baxteri)* and Canadian toads *(Bufo hemiophrys)* (Taylor, Williams, and Mills 1999; Taylor, Williams, Thorne, et al. 1999) previously attributed to *Basidiobolus ranarum* are likely due to infections with *Batrachochytrium.*

Colonization of the thickened epidermal layers with bacteria or other fungi is common and can result in secondary infections. An associated inflammatory response within affected skin is usually absent or minimal except in some caudates (Davidson et al. 2000) or in anurans with secondary bacterial or fungal infections as above.

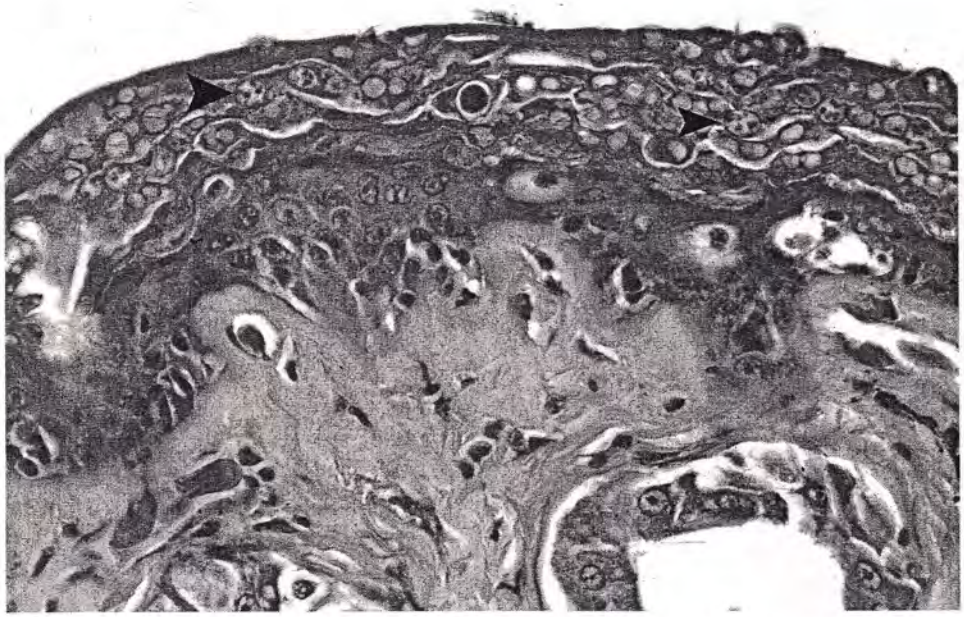

Figure 6-1 Histologic section of the skin from a White's treefrog (*Pelodryas caerulea*) showing myriad chytrid thalli (arrowheads) within the markedly thickened stratum corneum (external epidermal layers)

Diagnosis

Because the visual appearance of chytridiomycosis can mimic other amphibian diseases under some circumstances, the ideal samples to collect in any die-offs that are suspected to be caused by disease include those that would allow diagnosis of both chytridiomycosis and other conditions. These samples include formalin-fixed sections of skin and major internal organs, frozen samples of major internal organs or whole carcasses, and samples for routine bacteriology, if carcasses are fresh. Samples intended for histologic examination should not be frozen. If freezing of some samples is not possible at a field site, histologic examination of formalin-fixed tissue samples alone can provide the most information. Sections of skin, including the ventral abdominal skin (most preferably the pelvic patch), sections from the hindlimbs and feet, or whole carcasses are most important for diagnosis of chytridiomycosis. Even frozen and very decomposed tissue can be useful under many circumstances because the walls of chytrid thalli are hardy within the skin.

Diagnosis of heavy infections, which are those that are clinically significant or associated with die-offs, is usually straightforward. In such instances, histologic examination of skin sections by a veterinary pathologist or other trained individual will show characteristic tissue changes and chytrid thalli, as described above. Rapid diagnosis of heavy infections can be accomplished using smears or scrapings of skin air that is dried on microscope slides and stained with Romanovsky-type hematologic dyes (such as Diff-Quik) to reveal

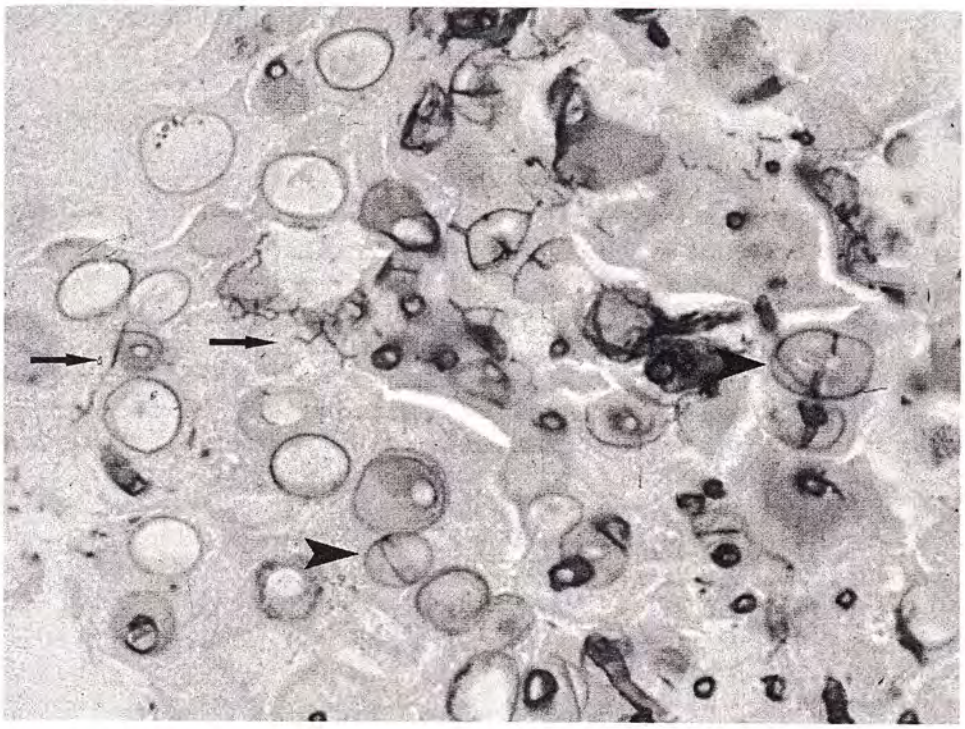

Figure 6-2 Silver-stained (GMS) histologic section of skin from a boreal toad (*Bufo boreas*) showing outlines of numerous chytrid thalli. The section includes internally septate colonial thalli (arrowheads) and fine branching rhizoids (arrows).

thalli (Pessier et al. 1999; Nichols et al. 2001). This technique is less reliable than histology, and results should be confirmed by histology. Alternatively, large areas of skin can be examined by making a mount of fresh skin in water. Surface skin cells, with their nuclei, can readily be differentiated from *Batrachochytrium* thalli, which can be identified inside surface cells by characteristic thallus forms.

Diagnosis of low-level or subclinical infections is more problematic because histology examines only a small surface area of the skin and may miss the characteristically clumped thalli of this stage. Examination of wet mounts of large areas of skin as described above may be helpful in some circumstances. Real time polymerase chain reaction (PCR) assays have been developed (A. Hyatt et al., Commonwealth Scientific and Industrial Research Organisation, Geelong, Victoria, Australia, personal communication) that can detect infection as early as one week post infection thereby overcoming the obvious shortfalls of the older diagnostic assays. Additionally, a PCR assay that uses primers that are specific to *B. dendrobatidis* is being developed (S. Annis et al., University of Maine, Orono, Maine, USA, personal communication).

B. dendrobatidis was identified as a chytrid by identification of reproductive spores characteristic of chytrids in electron photomicrographs (Pessier et al. 1999). Although the use of electron microscopy is not a necessary step in diagnosis, chytridiomycosis can also be confirmed with electron microscopy if the characteristic life stages are seen.

Pathogenesis

Chytridiomycosis is transmitted by zoospores, which are motile reproductive bodies formed inside mature thalli (sporangia). The zoospores settle on amphibian epidermis, enter the keratinocytes of the superficial layer, and form a thallus. Zoospores infect only keratinized tissue, which includes the skin of metamorphosed individuals, including adults. Infection is primarily on the ventral surface of animals, but thalli may be on the dorsal side in heavy infections. Infection in larvae is limited to the keratinized mouthparts or on the toe tips in late stages just prior to metamorphosis. The mechanism by which this pathogen causes death is unknown. Hypotheses are that death is caused by a toxin secreted by the fungus or that thickening of the skin results in disruption of cutaneous functions, such as osmoregulation and respiration.

Description of Batrachochytrium dendrobatidis

All current isolates of *Batrachochytrium* appear to be members of the same species, *B. dendrobatidis*. This species forms simple, more or less spherical, sporangia within the keratinized epidermal cells of amphibians. Zoospores are released through one or more discharge papillae, usually one when in amphibian skin, but more in the larger sporangia formed when the fungus grows in pure culture. The length of discharge papillae varies from negligible to tube-like. *Batrachochytrium* is distinctive in forming colonial sporangia. Cell walls form early in development in some thalli, and each cell develops into a sporangium. This type of development occurs in a fraction of thalli in pure culture (Longcore et al. 1999) and is responsible for an identifying character in slides stained for histological studies.

When the Chytridiales were last included in a monograph (Sparrow 1960), the taxonomy was based on morphological features that are not predictive for determining relationships. In other taxonomic groups, what is known for other members of the genus or family can be used to make predictions about a newly discovered species. However, this has not been possible in the case of *Batrachochytrium.* This new species did not fit into any known genus, either those known from classical morphological features (Sparrow 1960; Karling 1977) or those based on the ultrastructure of the zoospore (Barr 1980, 2000). Consequently, a new genus name was needed. Families in the Chytridiales are based on grades of development, rather than on attempts to predict phylogeny and thus are unhelpful in determining a near relative. Even phylogenetic trees based on analyses of sequences of the 18S rRNA gene of a variety of chytrids in culture (James et al. 2000) showed no close relatives for *Batrachochytrium.* Chytrids that grouped with *Batrachochytrium* in different analyses include an isolate (JEL 151) identified as *Rhizophydium haynaldi*, which was isolated from a cyanobacterium, but which also grows on pollen. The other is an unidentified chytrid (JEL isolate 142) from an acidic lake and was isolated from onionskin bait. Neither of these isolates is closely related or has enough in common with *Batrachochytrium* to help answer questions about its life cycle. Consequently, we have no

known close relative to look to for clues about whether *B. dendrobatidis* reproduces sexually or forms thick-walled resistant spores that can survive desiccation. *Batrachochytrium* reproduces asexually in culture and in frog skin. In the few years since its discovery, we have worked with this organism but have not seen any signs of sexual reproduction or of resistant spores. Sexual reproduction does occur in some members of the Chytridiales, and alternation of generations in different hosts is not uncommon in the chytrid order Blastocladiales. An investigation of the molecular genetics of North American, Australian, and Central American isolates suggests that *Batrachochytrium* reproduces clonally and that sexual reproduction is infrequent or unlikely (Morehouse et al. 2003).

Biological information about Batrachochytrium

Biological information about isolates of *Batrachochytrium* grown in pure culture can help in understanding the distribution of this pathogen in nature. If the morphology of different isolates were distinctive, we would hypothesize that we were dealing with several species of a genus. The morphology of *Batrachochytrium* in pure culture is plastic: Size, number of discharge papillae, length of discharge tubes, and sporangial shape vary with differences in microhabitat on a culture plate or within a container of broth. Although our isolates in culture come from a number of localities scattered throughout North America, the differences among isolates are not great and can be accounted for by differences in culture conditions. Type of development, shape of rhizoids, and generation time are consistent for all isolates that we have tested. If different species of *Batrachochytrium* exist, they have yet to be discovered or are cryptic.

The type isolate of *Batrachochytrium* came from a blue poison dart frog *(Dendrobates azureus)* from the National Zoological Park in the United States (Pessier et al. 1999). The optimal temperature for growth is 23 °C, and other isolates grow well at that temperature as well. At 28 °C to 29 °C cultures do not grow and die after extended incubation at 30 °C (Longcore et al. 1999). Studies are currently underway (Piotrowski et al. 2001) to confirm these temperatures with additional isolates and additional replications. When growing stock cultures, we incubate cultures of *Batrachochytrium* at 23 °C until growth is visible on the sides of the glass culture tube and then refrigerate them (ca. 6 °C). *Batrachochytrium* continues to grow slowly at this temperature, and cultures stay viable for up to 7 months.

Because *Batrachochytrium* grows in pure culture, it cannot be considered an obligate parasite. After amphibians have died from chytridiomycosis and populations have disappeared from a watershed, an essential question for managers is whether that watershed remains infective to reintroduced amphibians. At this time, it is not possible to detect *Batrachochytrium* in the field on keratin or other substrates used as bait, but the use of sentinel amphibians at a site might reveal whether the fungus is still present and infective. In the absence of amphibian hosts in a watershed, does *B. dendrobatidis* continue growing either on another host or saprobically on another substrate? In pure culture, *B. dendrobatidis* grows on other sources of keratin, such as snakeskin. Whether it can compete on this or other substrates in nature, however, is doubtful.

Why is chytridiomycosis now causing disease and mass mortalities of amphibians? One current hypothesis is that chytridiomycosis is an emerging infectious disease (Daszak et al. 1999). The disease occurs spottily in Europe and locally in New Zealand, and widespread declines in Australia and Central America are hypothesized to be spreading as a wave-like front. The earliest museum specimens that showed evidence of infection by *Batrachochytrium* were collected in the 1970s, about the time of the start of amphibian population declines in North America and Australia (Carey et al. 1999; Green and Sherman 2001). A test of the emerging infectious disease hypothesis by examination of the population biology of the fungus with molecular methods has yielded evidence that *B. dendrobatidis* is a clonally reproducing organism with little genetic variation (Morehouse et al. 2003). Alternative hypotheses, including the possibility that this chytrid has been present in many aquatic environments for some time and its emergence as a vertebrate pathogen has been triggered by one or more environmental stressors, have not yet been addressed.

Management implications

If chytridiomycosis is an emerging infectious disease, what can be done about it? Can actions be taken that will prevent its spread? The mechanism by which it is spread naturally is currently unknown. The Declining Amphibian Populations Task Force (DAPTF 2001) has issued guidelines about decontamination of gear and equipment between research sites, and conscientious individuals routinely treat boots, nets, buckets, etc. with 10% bleach between watersheds. On a broader scale, chytridiomycosis can be distributed geographically by human transport of infected amphibians. Animals from biological supply houses are frequently infected and should not be released into the wild. Importation of amphibians into areas free of the disease may need to be more strictly regulated than is the practice currently to prevent further distribution of chytridiomycosis.

Research needs

Many key questions about *Batrachochytrium* remain unanswered at this time. Do some amphibians act as vectors of chytridiomycosis? Anecdotal evidence suggests that different species of amphibians are differentially susceptible to infection by *Batrachochytrium*. Not all amphibian species die, or even become severely infected, when exposed to zoospores (Davidson et al. 2000; Parker et al. 2002; P. Daszak, Strieby, and Porter, Consortium for Conservation Medicine, Palisades, New York, USA, unpublished data), and different age classes react differently as well. Smaller individuals within the same species appear more susceptible than do larger ones (C. Carey et al., University of Colorado, Boulder, Colorado, USA, unpublished data). These observations mean that some sub-clinically infected species may serve as sources of disease propagules that may infect more susceptible hosts. Additional information is needed about how individual species react to infection so that their ability to act as vectors can be evaluated. Is *B. dendrobatidis* an introduced organism, and if so, where did it evolve? Several research groups are using different molecular methods to investigate the population biology of *Batrachochytrium*, but determining the geographic source of the infection may require extensive attempts to find the pathogen in the field and to isolate cultures from all conti-

nents. Finally, can the worldwide epidemic of chytridiomycosis be stopped? The fate of unexposed native amphibian populations worldwide may rest on the ultimate answer to this question.

Iridoviruses

Iridoviruses of the genus *Ranavirus*, first recognized in anurans in the 1960s (Granoff et al. 1965; Wolf et al. 1968), have increasingly been responsible for disease outbreaks in both anuran and caudate amphibians worldwide (Daszak et al. 1999; Hyatt et al. 2000). Frog Virus 3 (FV3) was the first iridovirus described in amphibians. We now know of many virulent amphibian ranaviruses that infect a wide range of hosts (Table 6-2), but the details of the host–pathogen biology of these hosts and viruses are generally poorly known.

Table 6-2 Ranaviruses (Family Iridoviridae) found in amphibians (does not include iridovirus-like erythrocytic or leukocyte viruses)

Virus	Host species	Origin of amphibians	General effects	References
Frog Virus 3 (FV3) [a]	*Rana aurora* (tadpoles; wild)	California, USA	Asymptomatic to death; associated with tadpole carcasses but not confirmed as the aetiological agent	Mao et al. 1997, 1999; Chinchar 2002
	Rana catesbeiana (tadpoles, metamorphosing juveniles and adults; wild and experimental exposure)	Throughout the USA: Wild populations in Alabama, Arkansas, North Carolina, and West Virginia; also in animals from biological supply houses in Illinois, North Carolina, and Wisconsin; and from a fishery in West Virginia, USA.	Asymptomatic to death; smaller & younger animals more susceptible	Clark et al. 1968; Wolf et al. 1968,1969
	Rana grylio (aquaculture colony)	Hubei, China. Exotic species reared for aquaculture. *R. grylio* is endemic to the USA but has been reared in several countries in Asia.	Mass mortality in aquaculture colonies	Zhang et al.1996, 1999, 2001
	Rana palustris (experimental exposure)	Unknown but species is endemic to eastern USA	Lethal in embryos	Granoff et al. 1965; Granoff 1969

continued

[a] Includes Lucke-*Triturus* virus (LT) 1–4, Redwood Creek virus, *Rana grylio* virus, *Rana tigrina* virus and tadpole edema virus (TEV) (Chinchar 2002)

Table 6-2 continued

Virus	Host species	Origin of amphibians	General effects	References
FV3 (continued)	*Rana pipiens* (wild and experimental exposures)	Wisconsin, Minnesota, and Vermont, USA; Saskatchewan, Canada	Asymptomatic to death; lethal in embryos, younger animals more susceptible	Clark et al. 1968; Granoff et al. 1965, 1966; Tweedell and Granoff 1968; Granoff 1969; D.M. Schock, Bollinger, and Chinchar, Arizona State University, Tempe, AZ, US, unpublished data
	Rana sylvatica (wild)	Eastern USA; Saskatchewan, Canada	Asymptomatic to death; lethal in embryos, younger animals more susceptible	Granoff et al. 1965; D.M. Scock, Bollinger, Chinchar, Arizona State University, Tempe, AZ, USA, unpublished data
	Rana tigrina (aquaculture colony)	Angthong, Ayuthaya, Nakonnayok, Nonthaburi, Pathumthani, and Singburi Provinces, Thailand. Species endemic to Southeast Asia	Mass mortality in aquaculture colonies	Kanchanankhan 1998; Zhang et al. 2001; Chinchar 2002;
	Bufo americanus (adults; experimental exposure)	Unknown but species is endemic to eastern USA	Mortality	Wolf et al. 1968
	Bufo marinus (tadpoles and adults; experimental exposure)	Queensland, Australia (species is endemic to South America but widely introduced)	Asymptomatic to death; tadpoles and juveniles appear more susceptible	Hyatt et al. 1998
	Bufo woodhousii fowlerii (tadpoles, juveniles, and adults; experimental exposure)	Lake Erie, Ontario, Canada and northeastern USA (collected from wild)	Asymptomatic to death	Clark et al. 1968, 1969; Wolf et al. 1968, 1969
	Scaphiopus intermontanus (adults; experimental exposure)	Unknown but species is endemic to western USA	Mortality	Wolf et al. 1968
	Notophthalmus viridescens (efts and adults; captive and experimental exposure)	North Carolina, South Carolina, and elsewhere in eastern USA (biological supplier)	Asymptomatic to death; younger animals more susceptible; virus also isolated from apparently healthy animals immediately upon receipt from supplier	Clark et al. 1968, 1969

continued

Table 6-2 continued

Virus	Host species	Origin of amphibians	General effects	References
Rana temporaria United Kingdom virus (RUK) [b]	*Rana temporaria* (juveniles and adults; wild)	Sussex, Essex, Hampshire, Surrey, Middleshire, and Dorset, United Kingdom	Mass mortality, possibly population declines	Cunningham et al. 1993,1995, 1996; Drury et al. 1995; Hyatt et al. 2000
Ambystoma tigrinum virus–Regina ranavirus (ATV–RRV) complex [c]	*Ambystoma tigrinum* (wild)	North Dakota and Utah, USA	Mass mortality	Green et al. 2002
	Ambystoma gracile (experimental exposure)	Washington, USA	Mortality	Jancovich et al. 2001
	Notophthalmus viridenscens (experimental exposure)	Pennsylvania, USA	Mortality	Jancovich et al. 2001
Spotted salamander, Maine (SsME) iridovirus [d]	*Ambystoma maculatum* (adults, wild)	Maine, USA	Mass mortality	Green et al. 2002
Guatopo virus (GV) [e]	*Bufo marinus* (adults; wild)	Guatopo National Park, Maturin, Cumana, and Margarita Island, Venezuela	None observed in wild animals	Zupanovic et al. 1998; Hyatt et al. 2000
	Bufo marinus (tadpoles, juveniles and adults; experimental exposure)	Queensland, Australia (exotic species)	Asymptomatic to death; younger animals more susceptible	Hyatt et al. 1998
	Leptodactylus sp. (adult; wild)	Maturin, Venezuela	None observed in wild animals	Zupanovic et al. 1998; Hyatt et al. 2000
	Litoria infrafrenata (metamorphs; experimental exposure)	Lab-reared; endemic to Australia	Asymptomatic to death	Hyatt et al. 1998
Bohle Iridovirus (BIV)	*Bufo marinus* (tadpoles, juveniles and adults; experimental exposure)	Queensland, Australia	Asymptomatic to death; younger animals more susceptible	Cullen et al.1995; Hyatt 1998.

continued

[b] Molecularly similar to FV3

[c] Closely related ranaviruses initially isolated from tiger salamander die-offs

[d] More similar to FV3 than to ATV–RRV complex

[e] Multiple isolates known, all from Venezuela

Table 6-2 continued

Virus	Host species	Origin of amphibians	General effects	References
BIV (continued)	*Limnodynastes ornatus* (wild)	Queensland, Australia	Asymptomatic to death; recent metamorphs more susceptible than tadpoles	Speare and Smith 1992; Hyatt et al. 1998
	Limnodynastes terraereginae (tadpoles and juveniles; experimental exposure)	Mount Spec, Australia	Asymptomatic to death; recent metamorphs more susceptible than tadpoles	Cullen et al.1995
	Litoria latopalmata (juveniles; experimental exposure)	Mount Spec, Australia	Asymptomatic to death	Cullen et al.1995
Bufo United Kingdom virus (BUK)	*Bufo bufo* (wild)	United Kingdom	Mortality	Hyatt et al. 2000
Rana esculenta iridovirus (REIR)	*Rana esculenta* (wild)	Croatia (various parts of the country, including two carp farms) and Romania	Unclear: found in moribund animals, but did not induce disease in experimental infection trials	Fijan et al. 1991; Ahne et al. 1998
Currently un-named or un-characterizied ranaviruses	*Ambystoma maculatum* (wild)	Massachusetts and North Carolina, USA	Mass mortality	USGS 2000
	Ambystoma tigrinum (wild)	Idaho, North Dakota, and Wyoming, USA	Mass mortality	USGS 2000
	Pseudacris crucifer (wild)	Maine (Acadia National Park); USA	Mass mortality	USGS 2000
	Rana catesbeiana (wild)	North Carolina, USA	Mass mortality	USGS 2000
	Rana septentrionalis (wild)	Minnesota, USA	Mass mortality	USGS 2000
	Rana sylvatica (wild)	Massachusetts and North Carolina, USA	Mass mortality	USGS 2000
	Numerous frogs and salamanders (wild)	Tennessee, USA	Mass mortality	USGS 2000

Case study

Most current research is focusing on a group of closely related ranaviruses (*Ambystoma tigrinum* virus–Regina ranavirus [ATV–RRV] complex) that cause tiger salamander (*A. tigrinum*) epizootics in western North America. As our understanding of the host–pathogen dynamics of the ATV–RRV complex improves, it will serve as a model for predicting the details of other host and iridovirus pairs. The following sections briefly summarize host and virus biology in tiger salamanders, occurrence of epizootics, and our ideas about the evolutionary ecology of the system.

Tiger salamanders are distributed across the U.S. and from southern Canada to central Mexico from sea level to 2800 m. These salamanders usually breed in winter, early spring, and occasionally in summer and within metapopulations using ponds, marshes, and earthen stock tanks. Eggs develop into aquatic larvae, most of which metamorphose in summer and overwinter in terrestrial burrows; mature salamanders return to ponds to breed. Hundreds of thousands of larvae may hatch, but often less than 1% survive to metamorphosis (Figure 6-3). In some populations of tiger salamanders, cannibalistic morph larvae develop; they have broad heads and enlarged vomerine teeth. In permanent aquatic habitats, larvae may forego metamorphosis, mature, and breed as a neotenic phenotype that remains in the water all year (Collins et al. 1993).

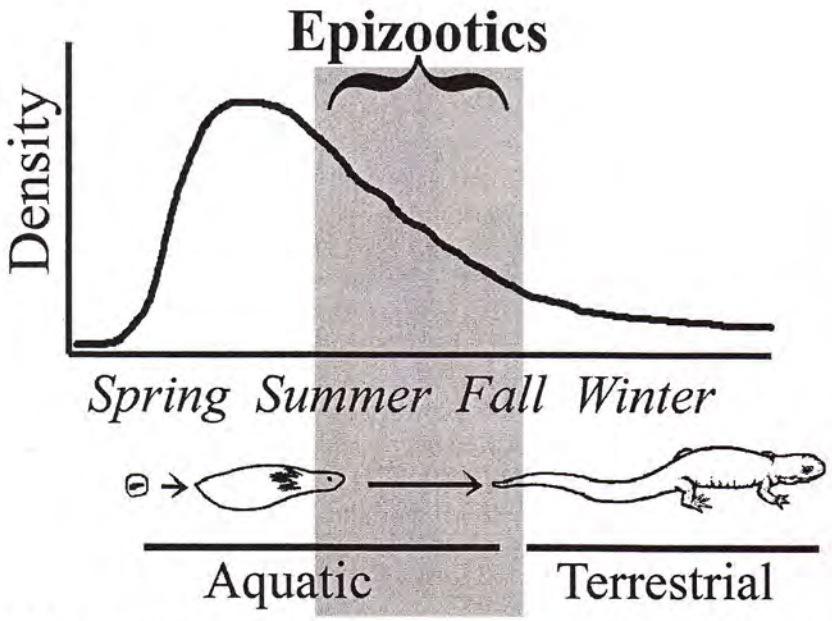

Figure 6-3 Generalized change in density of salamanders throughout a season. Tiger salamander densities fluctuate considerably in ponds, increasing rapidly with breeding in the early spring and then declining through the summer and fall due to predation, cannibalism, metamorphosis, etc. Though not represented in this figure, pond drying may increase the density of salamanders before causing significant mortality.

Epizootics

Episodic tiger salamander die-offs have been observed since 1985 in cattle tanks in the grassland and scrub oak forests of the San Raphael Valley (SRV) in southern Arizona, U.S.A. (Collins et al. 1988). The etiological agent of these die-offs, ATV, was isolated from dead and moribund salamanders (Jancovich et al. 1997). Koch's postulates were fulfilled, and ATV is consistently associated with SRV epizootics (J. Brunner and J. Jancovich, Arizona State University, Tempe, Arizona, USA, personal observation). Tiger salamander die-offs also occur in stock tanks, natural ponds, and marshes found in meadows and ponderosa pine forests of northern Arizona's Kaibab Plateau. An ATV-like ranavirus was isolated from carcasses in 2000 and 2001 (J. Brunner and J. Jancovich, Arizona State University, Tempe, Arizona, USA, personal observation). The manner in which the disease presented itself in these cases was similar to descriptions of infected animals in earlier Kaibab Plateau epizootics (Berna 1990; Pfenning et al. 1991), suggesting that a ranavirus also caused these die-offs. New methods for detecting ranavirus DNA in formalin-fixed tissues (Kattenbelt et al. 2000) will allow us to test for ranaviral infections using preserved salamanders from these earlier epizootics.

Regina ranavirus (RRV) was independently identified as the cause of four tiger salamander die-offs (separated by at least 50 km) in southern Saskatchewan, Canada, in 1997 (Bollinger et al. 1999). Each year since, two or more new sites of RRV-related die-offs have been identified in natural wetlands and artificial ponds in upland habitats, including non-irrigated cropland and native grass pastures.

Ranaviral epizootics are regular occurrences in some populations, but there is no simple pattern to their timing and frequency. Epizootics occur in successive years in some ponds (especially in Saskatchewan), but are separated by more than a decade in others. Epizootics are also widespread across landscapes. For example, in 1 year, die-offs occurred in 5 of 10 regularly sampled lakes on the Kaibab Plateau, which is about the size of Rhode Island, U.S.A. (Berna 1990), and 11 of 30 stock tanks in the SRV, which is about 26 km in length. In northern Arizona and Saskatchewan, which have distinct, severe winters, epizootics predictably occur in late summer and early fall (late June to early July in Saskatchewan and July through August on the Kaibab Plateau), although infected animals are sometimes found earlier in a season. Epizootics of neotenes occur throughout the year in the SRV.

Viral biology

Ongoing research that includes molecular characterization and nucleotide sequence analysis of the ranaviruses isolated from tiger salamander die-offs suggests that ATV and RRV are strains of the same viral species. ATV and RRV cause systemic infections that are not localized to a specific tissue (Jancovich et al. 1997; Bollinger et al. 1999) and are generally lethal within 11 to 17 days post-exposure (Jancovich et al. 1997). Transmission occurs via contact with infected salamanders or with water that holds infected salamanders.

The viral life cycle appears to occur within tiger salamanders; no syntopic, alternate hosts have been identified (Jancovich et al. 2001). Our recent research shows that these viruses are short-lived in the environment. Between epizootics, the virus appears to persist in sublethally infected, metamorphosed salamanders that presumably infect or reinfect other salamanders when they return to breed. At the end of an epizootic on the Kaibab Plateau, infected metamorphosed salamanders were observed leaving the pond for overwintering burrows. While it is unclear whether these animals will survive the winter and/or return to ponds still infected, in Saskatchewan virus was isolated from apparently healthy metamorphosed animals within 2 weeks of returning to breed. Additionally, in the laboratory, some infected salamanders recover but harbor transmissible, sublethal infections (Brunner et al. 2003).

Population effects of viral infection

The long-term effects of viral infections on salamander populations are uncertain. Tiger salamander die-offs can be extensive—sometimes overwhelming scavengers and decomposers with thousands of carcasses. One Kaibab Plateau population had 95% of a year class infected (Brunner et al. 2003); in other cases, entire year classes were killed (Berna 1990). The effects of an epizootic can be lasting, especially when die-offs recur. In frequently sampled Saskatchewan sites, no more than 10 tiger salamanders a year have been collected since a 1998 epizootic (D. Schock, Arizona State University, Tempe, Arizona, USA, personal observation). In other apparently healthy tiger salamander populations, RRV has been found at low frequencies throughout the activity season and up to 3 years after a die-off (D. Schock et al., Arizona State University, Tempe, Arizona, USA, unpublished data).

Evolution of tiger salamanders and iridoviruses

Three lines of evidence suggest that ATV and RRV have evolved with their tiger salamander hosts. First, sublethal infections persist in salamander populations. Second, ATV and RRV strains differ in vitro and in vivo in virulence, thermal preference, and disease phenology, all of which are consistent with local adaptation of host and pathogen, suggesting the system is old. Third, tiger salamander populations in Arizona and Saskatchewan appear stable (but see Alford and Richards 1999). The upper lethal limit of RRV isolated from Saskatchewan is 23 °C, while ATV isolated from southern Arizona grows at temperatures up to at least 31 °C (J. Jancovich, Arizona State University, Tempe, Arizona, USA, personal observation). A recent common garden experiment demonstrated that some strains are highly virulent, while others kill their hosts over a longer period of time (D. Schock, Arizona State University, Tempe, Arizona, USA, personal observation). These differences in virulence are host-dependent as well; salamanders from the population with the most virulent virus were most resistant to all viral strains.

It is uncertain if ranaviruses threaten the long-term persistence of tiger salamander populations, but it seems clear that they have the potential to do so. In particular, three anthropogenic effects on tiger salamander ecology make these viruses a significant threat. First, tiger salamanders captured for fishing bait and pets are moved considerable distances across geographic and political borders and are often released into native salamander

populations (Collins 1981). Introductions can lead to introgression of genes from exotic into native populations (Storfer et al. forthcoming). Bait shops keep their salamanders in densely stocked holding tanks that facilitate viral transmission, and these collections of larvae can be ideal conditions for amplifying viral virulence. In addition to making the virus more widespread, transportation of salamanders shuffles co-evolved hosts and pathogens, producing unpredictable population-level effects. Moving other salamander species, several of which are susceptible to ATV infection (Jancovich et al. 2001), can have the same effect. Viruses carried by fish may also infect salamanders. Twenty-eight days after injection, RRV was isolated from northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum*) that were experimentally infected (D.M. Schock et al., Arizona State University, Tempe, Arizona, USA unpublished data). Pike are often introduced for sport fishing, and while transmission from pike to salamanders has not been demonstrated, these fish must be considered a possible means for moving virus among salamander populations.

Second, humans are also changing the way in which aquatic habitats are organized on a landscape, making them more permanent (e.g., for watering cattle), more connected, and in some cases, more densely situated across a landscape. These changes would seem to benefit salamanders. But facilitating salamander movement has the inadvertent effect of increasing transmission of pathogens, and in theory, well-connected metapopulations are also able to support more virulent pathogens. Thus, by altering the distribution and configuration of ponds we may inadvertently be selecting more virulent viruses.

Third, there clearly must be an interaction between a salamander's immune defenses and the virus. Any factor that compromises immune function increases risk of mortality. Many potential stressors associated with global change may operate on a large enough scale to cause regional declines.

Pathology of amphibian iridovirus infection

Ranaviral infections in anurans most often occur in tadpoles and metamorphosing and recently metamorphosed animals. Experimental infections of Great Basin spadefoot toads (*Scaphiopus intermontanus*), American toads (*Bufo americanus*) and Fowler's toads (*Bufo fowlerii*) with tadpole edema virus (Wolf et al. 1968), marine toads *(Bufo marinus)* experimentally infected with Bohle iridovirus (Cullen et al. 1995), and natural infections of common frogs *(Rana temporaria)* from the United Kingdom (Drury et al. 1995) suggest that infections of adult animals can occur under some circumstances. Experimental evidence with *Limnodynastes terraereginae* exposed to Bohle iridovirus demonstrated that metamorphs were more susceptible to disease than tadpoles, suggesting that disease can depend on life stage (Cullen et al. 1995). In caudate amphibians such as tiger salamanders infected with RRV, disease has been observed in both larval and adult animals (Bollinger et al. 1999).

Clinical signs and gross pathology

Clinical signs and gross pathology of iridovirus infection in anurans can range from death without external manifestations, as observed with experimental Bohle iridovirus infections (Cullen et al. 1995) to combinations of externally visible hemorrhage (manifested

as skin reddening or multiple red spots) and edema (fluid accumulation within the skin or coelomic cavity) (Wolf et al. 1968). Bullfrog *(Rana catesbeiana)* tadpoles infected with tadpole edema virus (TEV) exhibited variable degrees of edema beneath the skin as well as diffuse hemorrhage of the ventral skin. Adult toads affected with experimental TEV infection were lethargic prior to death, with multifocal hemorrhages on the skin of the ventral hindlimbs. Necropsy demonstrated ascites (edema within the coelomic cavity) and small multifocal hemorrhages within the digestive tract and kidneys. Hemorrhages involving skeletal muscle have been described in adult *Bufo* spp. infected with TEV and adult *R. temporaria* (Wolf et al. 1968; Drury et al. 1995; Daszak et al. 1999). Ulcerative skin lesions were observed in *R. temporaria* (Drury et al. 1995; Daszak et al. 1999). In tiger salamanders affected with RRV or ATV, clinical signs were variable and ranged from sudden death to combinations of lethargy, anorexia, and loose or bloody stools (Jancovich et al. 1997). Skin lesions were variably observed in both RRV and ATV infections and consisted of pale raised foci (described as "polyps" with ATV) as well as areas of cutaneous erosion, ulceration, and hemorrhage; excessive skin mucus production was observed in some animals with RRV and ATV. Gross necropsy of animals with RRV frequently revealed ascites and petechiation (small hemorrhages, appearing as multiple red spots) of the surfaces of internal organs. Edema and hemorrhage were occasionally noted within the stomach wall (Bollinger et al. 1999).

It is important to note that these clinical signs can mimic the classically described "redleg" disease of amphibians, most often attributed to the gram-negative bacterium *Aeromonas hydrophilia* (Hubbard 1981), or skin diseases such as chytridiomycosis (Berger et al. 2000). Care should be taken not to diagnose redleg disease without correlating the results of bacterial culture with histology and possibly results of more specific viral diagnostics as described below because *A. hydrophilia* is a common contaminant and secondary pathogen in amphibians with confirmed viral infection (Wolf et al. 1968; Hird et al. 1981; Jancovich et al. 1997; Bollinger et al. 1999)

Microscopic pathology

Histologic examination of anuran and caudate amphibians shows similar microscopic lesions consisting primarily of areas of necrosis (cell death) and hemorrhage in multiple tissues, particularly the hematopoietic tissues, liver, digestive tract, and skin, all indicating a systemic infection. In experimental TEV infection of Great Basin spadefoot and American and Fowler's toads, lesions were described as vascular endothelial necrosis of the kidney, digestive tract, lung, fat body, and bladder with resultant hemorrhage (Wolf et al. 1968). Necrosis of renal interstitial tissue (presumptively renal hematopoietic tissue) was also described. As described for gross pathology, extensive hemorrhage was observed histologically in the muscles of *Bufo* spp. affected with TEV. *Limnodynastes terraereginae* and *Litoria latoplamata* experimentally infected with Bohle iridovirus had prominent areas of necrosis within the kidney, liver, stomach, spleen, and lung (Cullen et al. 1995). Necrosis within the kidney involved both renal tubules and glomeruli, as well as the interstitial hematopoietic tissue, and was associated with variable degrees of interstitial hemorrhage. As with anuran iridovirus infections, tiger salamanders with RRV infection had areas of necrosis (single cell to focal) within multiple tissues, with the exception of the nervous system and skeletal muscle (Bollinger et al. 1999). The most severe

histologic lesions were observed in the skin, gastrointestinal tract, liver, and hematopoietic tissue. Skin lesions consisted of areas of intercellular and intracellular edema of the stratum spinosum (a middle layer of the epidermis), with areas of degeneration progressing to subcorneal (beneath the outer layer of the epidermis) and intraepidermal clefts and vesicles and, ultimately, to epidermal erosions and ulcers. Intracytoplasmic amphophilic to basophilic inclusion bodies were commonly observed within affected tissues. Inclusion bodies are also observed in epizootic haematopoietic necrosis virus (EHNV) infection of fish, which is also caused by a *Ranavirus* (Reddacliff and Whittington 1996). Intranuclear inclusion bodies were also presumptively identified in tiger salamanders infected with ATV (Jancovich et al. 1997).

Diagnosis

Routine diagnosis of amphibian iridovirus infection is ideally based on a combination of clinical signs and gross pathology, if present. These methods should be combined with histologic analysis and confirmation with techniques such as transmission electron microscopy, virus isolation in cell culture (Wolf et al. 1968; Hyatt et al. 2000), immunologic techniques (Cullen et al. 1995; Hyatt et al. 2000), and nucleic acid–based techniques (Cullen et al. 1995; Hyatt et al. 2000; Kattenbelt et al. 2000). In field situations, in which it is not always possible to acquire fresh material, diagnosis can be based on histologic lesions (greatly simplified if intracytoplasmic inclusion bodies are present) followed by transmission electron microscopy to demonstrate characteristic icosahedral iridovirus virions. Immunohistochemical (Cullen et al. 1995) or polymerase chain reaction (PCR)–based methods (Kattenbelt et al. 2000) could be used to confirm diagnosis from paraffin-embedded histologic material. However, reagents for these techniques are not yet widely available. If fresh frozen materials can be obtained, virus isolation can be attempted using fish cell lines such as epithelioma papilloma cyprini (EPC) or bluegill fry (BF2) using described techniques described elsewhere (Wolf et al. 1968; Speare and Smith 1992; Jancovich et al. 1997; Bollinger et al. 1999; Hyatt et al. 2000). Identification in cell culture is based on observation of cytopathic effects followed by demonstration of iridovirus virions by negative stain electron microscopy or by application of antigen capture enzyme-linked immunosorbent assay (ELISA) (Hyatt et al. 2000).

Pathogenesis

Little is known about the precise pathogenesis and development of disease in amphibian iridovirus infections. Death is presumptively due to organ failure as a result of tissue necrosis of multiple organs. Speculatively, there is the additional possibility that necrosis of hematopoietic tissue could predispose infected animals to the development of secondary infections. In the laboratory, amphibian iridovirus infections can be transmitted by infected food (Wolf et al. 1968), feeding of tissues from infected individuals (Jancovich et al. 1997), injection (Wolf et al. 1968; Cullen et al. 1995), water previously housing diseased animals (Jancovich et al. 1997), feces (Moody and Owens 1994), and inadvertent laboratory transmission by handling (Jancovich et al. 1997). Isolation of virus from apparently unaffected animals suggests that carrier states can occur (Wolf et al. 1968).

Role of Invasive Predators and Competitors in Causing Amphibian Population Declines

An invasive animal species is regarded as an animal taxon that has become established in new areas as a result of human intervention (term modified from Sandlund et al. 1999). Other terms that have been applied to such taxa include "exotic," "introduced," "alien," and "nonnative." In addition to invasive animal species, other invasive organisms, such as plants, can adversely affect amphibian populations and communities and are addressed in Chapter 7. Invasive animal species have been implicated as causal agents in population declines of numerous amphibian species, including species in North America, Central America, and South America; Europe; Asia; and Australia (Kuzmin 1994; Alford and Richards 1999; Gillespie and Hero 1999; Corn 2000; Semlitsch 2000). For example, invasive species have been implicated as adversely affecting populations of at least 23% of 91 native anurans in the U.S., including several threatened or endangered species (Bradford forthcoming). Such invasive species include numerous fishes, the American bullfrog (*R. catesbeiana*) and other amphibians, crayfishes, and defoliating insects. These invasive species may variously affect native amphibians as predators, competitors, agents of habitat change, genetic biopolluters (through hybridization), and vectors of disease. These consequences were not intentional but have resulted almost universally from anthropogenic activities: deliberate introductions for sport fishing or pest control; escape of animals from commercial enterprises, such as bullfrog aquaculture, laboratory testing, or the pet trade; use as fish bait; and transportation of plant products.

Two major challenges confront researchers and resource managers in rectifying this situation. The first is to establish conclusively whether an invasive species is indeed responsible for population declines of amphibians. Such conclusive studies establishing cause and effect should not be a prerequisite to taking action to address an environmental problem. Otherwise natural resources will continue to be lost because "[t]ight causal proofs are elusive in ecology as in most science" (Woodwell 1989). However, firmly establishing cause and effect is often necessary before any major action is initiated by resource managers to reverse the impacts of invasive species. Agency actions may require changing a long-standing policy with a substantial public constituency, such as those supporting fish stocking and insect pest control. Moreover, the problem may be viewed as intractable, and the proposed solutions may be costly. For resource managers, it is usually not sufficient to know that a given factor may contribute to a certain problem. They need to know that eradication of the suspected factor can be expected to result in an appreciable alleviation of the problem. Regardless of an agency's intentions, uncertainty about the cause of the problem is likely to lower its priority for action relative to the many other environmental concerns on the agency's agenda.

The second major challenge is to develop the necessary approaches and tools for restoring a system. Efforts to eradicate or control invasive species affecting amphibian populations are relatively recent endeavors. Although this task is clearly difficult at present in many circumstances, some recent success stories are encouraging.

The challenge to establish cause and effect

Unfortunately, conclusive studies are lacking for most systems where invasive taxa are thought to be affecting amphibian populations. At present, most inferences about the negative effects of invasive species on native amphibians are based on anecdotal observations or scant data, with little evaluation of alternative hypotheses. This state of affairs exists because of a number of difficulties, aside from limited study. For example, in many cases the invasion by the non-native species has occurred concomitantly with changes in other anthropogenic or natural factors, such as land use and hydrological regimes (Hayes and Jennings 1986). Moreover, data are typically scant for the distribution and abundance of the native species prior to the apparent replacement. Also, in some cases the population decline of the native species has been so extensive that there are virtually no sites available to examine the invasive–native interaction (Corn 2000).

Several types of information or studies have been used to evaluate putative cause-and-effect relationships between invasive species and native amphibians. Each of these has potential values and shortcomings in determining cause and effect. In general, a combination of these studies is necessary to provide a convincing and clear understanding for the role of invasive species in causing amphibian population declines. The types of studies include the following discussed below.

Anecdotes

These are typically observations of the replacement of native species by invasive taxa at a few sites over time or observations of invasive taxa occupying sites thought to be suitable habitat for the native species. Such observations have been instrumental in developing concern for the native species, but they do not provide an adequate basis to establish cause and effect. In somewhat surprising contrast, however, anecdotes can prove quite powerful in contradicting substantial, quantitative data (see "Case study for establishing cause and effect: The mountain yellow-legged frog in Sierra Nevada," p 184).

Univariate analyses of distribution or abundance

These studies evaluate the relationship between the distribution or abundance of the native species relative to a single dependent variable (i.e., the distribution or abundance of the invasive species). Many studies have demonstrated a pattern of complementary distribution (i.e., allotopy), in which invasives and natives generally do not occur together in the same place (e.g., Hayes and Jennings 1986; Bradford 1989; Fisher and Shaffer 1996; Gamradt and Kats 1996; Hecnar and M'Closkey 1997; Adams 1999; Funk and Dunlap 1999). Other studies have demonstrated an inverse relationship between the abundances of the invasive and native species at sites where they both occur (e.g., Tyler, Liss, Ganio, et al. 1998). Such patterns are consistent with the hypothesis that the invasive species has had a substantial deleterious effect on a native species. These findings by themselves, however, do not establish cause and effect because alternative hypotheses (e.g., habitat differences and other anthropogenic stressors) are not substantially addressed in the analyses. Nevertheless, these analyses can provide statistically valid tests of predictions, and when sites are sampled over a large area, they can provide valuable information about the geographic extent of the putative problem.

Multivariate analysis of distribution or abundance

These studies improve upon univariate analyses by including independent variables for other factors that potentially could influence the distribution or abundance of the native species, including variables that may reflect multiple, anthropogenic stressors (Adams 1999; Knapp and Matthews 2000; Pilliod and Peterson 2001). Such studies must be designed to have sufficient power to detect patterns among the multiple variables, which generally necessitates a large sample size of sites. These studies can provide strong evidence for or against invasive species as causal factors for population declines, especially when hypotheses are tested at multiple spatial scales or in multiple areas (Adams 1999; Knapp and Matthews 2000; Pilliod and Peterson 2001).

Addition or removal studies

Well-controlled, replicated field experiments adding or removing invasive or native taxa to or from a system have the potential to provide the most convincing data for cause and effect. Such studies, however, are typically limited by a number of circumstances. For example, it may not be desirable to introduce an invasive species to areas that it has not yet invaded, and the removal of invasives from a site may not result in recolonization by the native because extant populations no longer exist nearby. Moreover, the removal of the invasives can be very labor intensive or otherwise problematic (e.g., bullfrogs; Rosen and Schwalbe 1995). Nevertheless, recolonization of sites has been observed for some amphibians following extinction of the introduced fish population (Funk and Dunlap 1999; Knapp, Matthews, and Sarnelle 2001). In those few instances in which fish populations were removed experimentally to evaluate the effect on frog populations, the manipulation resulted in rapid increases in frog population size (*Hyla arborea* [Brönmark and Edenhamn 1994]; *Rana muscosa* [V. Vredenberg, University of California Berkley, Berkley, California, USA, unpublished data; R.A. Knapp, University of California, Santa Barbara, Santa Barbara, California, USA, unpublished data]). Rosen et al. (forthcoming) introduced Chiricahua leopard frogs (*Rana chiricahuensis*) into sites with and without bullfrogs, and observed population persistence only in bullfrog-free sites. A major limitation of such studies is that usually only a few sites can be practically investigated.

Interaction experiments

A number of studies have been done to determine the modes of interaction between invasive species and native amphibians, such as predation or competition (e.g., Gamradt and Kats 1996; Gamradt et al. 1997; Kupferberg 1997; Kiesecker and Blaustein 1998; Tyler, Liss, Hoffman, and Ganio 1998; Gillespie and Hero 1999; Lawler et al. 1999). Such interactions can differ between the invasive and native species, depending on life stage and environmental conditions. These studies can elucidate plausible modes of negative interactions among the invasive and native species that may have significant effects at the population level. Moreover, insight gained in these studies about the biology of the interaction may facilitate the design of mitigation and restoration approaches (Lawler et al. 1999). Nevertheless, interactions observed in the laboratory, mesocosms, or controlled field situations cannot determine whether the exotics are the cause for population declines in the field.

Other approaches

Well-documented observations before, during, and after invasion of multiple sites would provide a strong empirical basis for assessing cause and effect. However, the timing of invasion is seldom predicted in advance, and research designed to capture such events is a gamble. A modeling approach for predicting site occupancy could also be helpful, especially for explaining why invasives and natives occur together in some circumstances, but not in others. In the case of invasive taxa interbreeding with a native species, population genetic studies are necessary to determine the magnitude of genetic change in the population (e.g., Storfer et al. forthcoming).

In sum, anecdotes, univariate analyses of distribution or abundance, and interaction experiments can contribute much to understanding the situation, but they are inadequate for firmly establishing the role of invasive species as agents of amphibian population declines. Multivariate distribution or abundance studies and addition or removal experiments have much greater potential to evaluate the role of invasives in causing declines, particularly when conducted at enough sites and in a diversity of circumstances to establish the generality of the findings. The most convincing arguments are based on weight of evidence using multiple studies of multiple types.

Case study for establishing cause and effect: The mountain yellow-legged frog in the Sierra Nevada

The mountain yellow-legged frog (*R. muscosa*) in the Sierra Nevada of California and Nevada, USA, formerly ranged throughout the mountain range above about 1500 m (Zweifel 1955; Jennings and Hayes 1994). It was frequently encountered at high-elevation lakes and streams and once considered the "commonest amphibian" in the Yosemite region (Grinnell and Storer 1924). Today, its distribution is greatly restricted, occurring at no more than 50% of its former localities in the Sierra Nevada (Jennings 1996; USFWS 2003). Substantial declines of populations have been observed beginning in the early 1900s and continuing to the present day, although the severity of these declines was not well appreciated until the 1980s and 1990s (Hayes and Jennings 1986; Bradford et al. 1994; Jennings and Hayes 1994; Drost and Fellers 1996; Jennings 1996; USFWS 2003). This dramatic decline has been striking in part because most of the range of the species occurs in national parks or wilderness areas, where environmental change over large areas is not evident. The species is currently a candidate for listing as an endangered species under the Endangered Species Act (USFWS 2003).

Of the many hypotheses for this dramatic decline (described below), invasive species were a concern from the outset (Grinnell and Storer 1924; Hayes and Jennings 1986). Despite this insight, it has taken an enormous amount of observation, research, discussion, and legal activity to spur agency action.

The rationale for the invasive fish hypothesis is fairly simple. The historic geographic range of *R. muscosa* coincided roughly with the glaciated portion of the Sierra Nevada (Zweifel 1955). This area was largely fishless prior to colonization by Europeans because waterfalls and cataracts prevented upstream colonization when the glaciers retreated (Knapp 1996). Beginning in the late 1800s, trout (*Salmo* spp., *Onchorhynchus*

spp., and *Salvelinus* spp.) have been stocked by foot, pack animals, automobile, and aircraft into thousands of water bodies (Knapp 1996). Subsequently, fish populations spread from these water bodies, resulting in occupancy of nearly all of the deeper water bodies (i.e., >3 m deep, 2 ha in area [Bahls 1992]). *R. muscosa* is largely restricted to lakes and streams with permanent water because tadpoles require up to several years to reach metamorphosis (Zweifel 1955). Adults also use permanent water for overwintering under ice (Bradford 1983; Matthews and Pope 1999; V. Vredenberg, University of California Berkley, Berkley, California, USA, personal communication). Consequently, the habitat requirements for *R. muscosa* and fish are largely the same (Bradford 1989; Knapp and Matthews 2000). Predation on amphibian larvae (Hayes and Jennings 1986) and occasionally adults is the presumed mode of impact by trout, although the transmission of disease by hatchery-reared fish has also been postulated (Kiesecker et al. 2001).

The first reports of an effect of invasive fishes were made by Grinnell and Storer (1924), who observed in the early 1900s that *R. muscosa* populations disappeared soon after lakes were stocked with fish. Needham and Vestal (1938) observed trout preying on *R. muscosa* in a lake into which trout had been recently introduced, and Cory (1963) described the virtual lack of co-occurrence between fish and frogs, except in ecologically complex systems. Common lore among fishermen held that the presence of *R. muscosa* tadpoles in open water of a lake indicates that the lake is barren of fish (Zardus et al. 1977). This implied allotopic distribution of *R. muscosa* and salmonids was later quantified in separate areas using univariate analyses by Bradford (1989) and Bradford et al. (1998), who argued that the introduction and spread of salmonids has resulted in the demise of *R. muscosa* at nearly all sites containing fish. Bradford et al. (1993) presented data that the distribution of fish has become so extensive that the remaining frog populations are largely isolated from one another at the heads of watersheds. Consequently, the probability of extinction at these sites is expected to be high, and the probability of recolonization from other frog populations following extinction should be low (Hanski and Gilpin 1991). Thus, the effects of invasive fish on *R. muscosa* populations may extend to waters not inhabited by fish, a phenomenon subsequently documented for the Columbia spotted frog (*Rana luteiventris*) and long-toed salamander (*Ambystoma macrodactylum*) in an Idaho mountain system (Pilliod and Peterson 2001). The role of invasive fishes on the distribution of *R. muscosa* was more rigorously tested using multivariate analyses in a study of more than 2000 lakes at 3 spatial scales by Knapp and Matthews (2000). Their study included fish and a number of natural environmental factors to evaluate whether fish or other factors were associated with the distribution of *R. muscosa*. They demonstrated that the presence or absence of fish was the most important factor determining the distribution of *R. muscosa*, and this effect was evident at the 3 spatial scales. Fish removal studies have corroborated the adverse effects of fish. Knapp, Matthews, and Sarnelle (2001) documented numerous cases in which *R. muscosa* recolonized a lake following extinction of the invasive fish populations due to cessation of trout stocking. More recently, fish have been removed using gill nets from 3 lakes where extant *R. muscosa* populations were located nearby, and 1 lake in another area where *R. muscosa* inhabited a portion of the lake. In the former case, frogs quickly immigrated from nearby populations, resulting in successful breeding and subsequent recruitment (V. Vredenberg, University of California Berkley, Berkley, California, USA, unpublished data). In the latter case, the number of

frogs and tadpoles increased approximately 20- and 100-fold, respectively, over the subsequent 4 years, an increase well beyond the population fluctuations for other populations in the region (R.A. Knapp, University of California Santa Barbara, Santa Barbara, California, USA, unpublished data).

When results of these exhaustive studies were used as evidence that invasive fishes have been a major cause for the dramatic population declines of *R. muscosa* in the Sierra Nevada, they were often met with counter arguments by resource management personnel. Foremost among these was that the fish hypothesis could not account for all declines, such as those in the Tableland area of the Kaweah River watershed, where fish are largely absent (Bradford 1983). This counter argument assumes that lack of universality negates demonstrated impacts elsewhere. Along a similar vein, anecdotal observations of co-occurrence of fish and frogs in some lakes were viewed as evidence for a non-effect by fish. Again, the argument assumes that lack of universality negates a statistically supported, general pattern. Doubt was also instilled because no less than 8 additional hypotheses were considered plausible for adversely affecting *R. muscosa* populations over wide areas: acidic deposition, disease, increased UV-B, climate change, extreme weather, livestock grazing, airborne pesticides, and airborne endocrine disrupter compounds (Bradford 1983; Bradford et al. 1992; Stebbins and Cohen 1995; Drost and Fellers 1996; Carey et al. 1999; Sparling et al. 2001; Davidson et al. 2002; USFWS 2003). In contrast, given that none of these hypotheses is mutually exclusive with the invasive fish hypothesis, concern about invasive fish should have been heightened by these possibilities rather than diminished by them. That is, if any of these other factors have caused population declines of *R. muscosa*, remedies for the offending factor would be unlikely to result in recolonization of the site by frogs from elsewhere because of the ubiquity of fish in intervening waters.

Presumably as a result of these studies and the petition to list the species as endangered in 1999, the California Department of Fish and Game, the agency with primary responsibility for management of fishes in the Sierra Nevada, suspended aerial fish stocking in some backcountry areas in 2001 pending further evaluation and embraced a policy of planning at the watershed scale. Although these steps are significant in ending the continual exacerbation of the problem, it is only a beginning for addressing the problem. Also in 2001, the U.S. National Park Service (USNPS) began an effort to eradicate non-native fish from selected areas in Sequoia and Kings Canyon National Parks (USNPS 2001). The National Park Service had earlier terminated fish stocking in Sierra Nevada national parks (between 1977 and 1990), largely because of the inconsistency between fish stocking and the mandate to preserve natural ecosystems in national parks.

Evidence for cause and effect in other cases

Many valuable observations and studies implicate invasive species as agents contributing to amphibian population declines, or as threats to extant populations. However, the role of invasive species in this action has been quantitatively addressed in relatively few cases. In general, data are scant for the distribution and abundance of the affected native species prior to the invasion, and other anthropogenic or natural environmental factors have changed concomitantly with the invasion. Moreover, in some cases the adverse effects of

invasive species collectively may be strongly implicated, but the role of individual taxa are not distinguished. For example, distributional data indicate that a suite of 15 invasive vertebrate species (13 fishes, American bullfrog, and tiger salamander [*A. tigrinum*]) are largely responsible for numerous population extirpations of the Chiricahua leopard frog (*R. chiricahuensis*) in southeastern Arizona (Rosen et al. 1995). Below is a discussion of some of the evidence for various invasive taxa adversely affecting amphibian populations.

As exemplified by the case study above, native amphibians have been particularly affected by invasive species in mountain lakes and streams, where predatory fish were originally largely absent but have been introduced for sport fishing. This fact applies to most of the mountain ranges in western North America, and portions of Central and South America, Europe, and Australia, where salmonids have been introduced (Braña et al. 1996; Gillespie and Hero 1999; Knapp, Corn, and Schindler 2001). Like *R. muscosa*, the effects of salmonids on the long-toed salamander in the Pacific Northwest has been well studied and has included univariate analyses, multivariate analyses, removal studies, and interaction experiments (e.g., Liss et al. 1995; Tyler, Liss, Ganio, et al. 1998; Tyler, Liss, Hoffman, and Ganio 1998; Pilliod and Peterson 2001; Pilliod and Fronzuto forthcoming). For example, in a multivariate study, the abundance of *A. macrodactylum* was inversely related to occupancy of lakes by fish at two spatial scales, individual lakes and basins (Pilliod and Peterson 2001), and colonization of lakes has been observed to follow extinction of introduced trout quickly (Funk and Dunlap 1999). Nevertheless, there is currently no evidence for change in the geographic distribution of the species (Pilliod and Fronzuto forthcoming). Some other taxa with evidence for adverse effects of introduced salmonids on populations include a number of *Rana* species (Hayes and Jennings 1986; Pilliod and Peterson 2001), *Pseudacris regilla* (Matthews et al. 2001), and *A. tigrinum* in North America (Corn et al. 1997); *H. arborea* and an amphibian community in Europe (Brönmark and Edenhamn 1994; Braña et al. 1996); and *Litoria spenceri* in Australia (Gillespie 2001).

Non-salmonid fishes of many species have also been implicated in amphibian population declines (Hayes and Jennings 1986; Gillespie and Hero 1999). For example, in the Central Valley of California, where population declines of several native amphibians have been pronounced, distributional data have implicated invasive species as a primary causal factor, although the effects of invasive fishes could not be separated from invasive bullfrogs (Fisher and Shaffer 1996). Among 178 ponds in southwestern Ontario that collectively contained 13 species of introduced predatory fish and 12 species of native amphibians, amphibian species richness was significantly lower at ponds having predatory fish than at non-predatory, or fish-free, ponds (Hecnar and M'Closkey 1997). Using multivariate analyses, Adams (1999) found that habitat structure and invasive predatory fish were significant determinants of the occurrence of the red-legged frog in northwestern Washington. Distributional data and a removal experiment supported the hypothesis that invasive fishes are an important factor in the reproductive success and occurrence of the treefrog *H. arborea* in Sweden (Brönmark and Edenhamn 1994). A number of studies have specifically addressed the potential effects of the mosquitofish (*Gambusia* spp.) on native amphibians. Gamradt and Kats (1996) reported the disappearance of the California

newt (*Taricha torosa*) from a stream following mosquitofish invasion and documented predation on newt tadpoles by mosquitofish in field experiments. Evidence for mosquitofish having a major impact on the distribution or abundance of Australian amphibians is equivocal. Several studies have shown allotopic distribution or reduced numbers in the presence of mosquitofish; however, others have not, and alternative hypotheses such as changes in habitat conditions have not been well considered (Gillespie and Hero 1999). Nevertheless, a number of experimental studies have shown that mosquitofish are capable of killing or injuring the tadpoles and eggs of a variety of amphibian species (Gillespie and Hero 1999).

A substantial body of literature implicates the American bullfrog, a species introduced to many areas in western North America, Europe, and Asia, as a cause for population declines of a number of native amphibians (e.g., Moyle 1973; Bury and Whelan 1984; Kupferberg 1997; Adams 1999; Casper and Hendricks forthcoming). For example, a number of investigators have documented a negative association between the distribution of native amphibians and invasive bullfrogs in permanent water habitats, suggesting adverse effects (Kupferberg 1997; multiple references in Adams 1999; Kiesecker et al. 2001). Frequently, studies addressing the effects of invasive bullfrogs on native amphibian populations have been problematic because of the difficulty in separating the effects of bullfrogs from invasive fishes and habitat change (Hayes and Jennings 1986; Kiesecker and Blaustein 1998; Adams 1999). Moreover, bullfrogs have been shown to have a strong impact on native frogs in some cases, but not others. A compelling case is with the Chiricahua leopard frog (*R. chiricahuensis*) in southern Arizona. Rosen et al. (forthcoming) documented the allotopic distribution between bullfrogs and *R. chiricahuensis*, a pattern distinct from the allotopic distribution between invasive centrarchid fishes and *R. chiricahuensis*. They further demonstrated that *R. chiricahensis* introduced to sites with and without bullfrogs persisted only in bullfrog-free sites. In contrast, in a multivariate study designed to distinguish among the effects of multiple factors on red-legged frog populations in western Washington, negative associations between bullfrogs and red-legged frogs were weak or absent, and greater significance was found for invasive fishes than for bullfrogs. In both southeastern Arizona and western Washington, habitat modifications were generally favorable to the invasive species and unfavorable to the native frogs (Adams 1999; Rosen et al. forthcoming).

A number of other invasive amphibians have also become established outside their native ranges in various parts of the world. For example, six species with ranges outside the U.S. have become established in the U.S. (Meshaka forthcoming; Hero and Stoneham forthcoming). One of these, the African clawed frog (*Xenopus laevis*) has also become established in Europe, Chile, and Ascension Island (Crayon forthcoming). It preys on native anurans, and clawed frog densities can reach remarkable numbers; however, adverse effects on native amphibian populations have not been demonstrated (Crayon forthcoming). Similarly, the marine toad (*B. marinus*) from the tropical Americas has been introduced to other tropical regions in a misguided effort to control rats or sugar cane pests, including Florida, Caribbean islands, Philippines, Fiji, New Guinea, and Australia (Hero and Stoneham forthcoming). In Australia, marine toads have dramatically affected the populations of various reptiles, mammals, and beetles, primarily due to the toxic effect of all

life stages of the toad when taken as prey (Hero and Stoneham forthcoming). Although many species of amphibians succumb to feeding on marine toad eggs, adverse effects of the marine toad on native amphibian populations in Australia have not been substantiated (Hero and Stoneham forthcoming). In the U.S., adverse effects are suggested for some ponds in Florida in which native amphibian populations declinded precipitously when *B. marinus* invaded (Punzo and Lindstrom 2001). Some U.S. amphibians other than bullfrogs have expanded their geographic ranges either through introduction to new areas or range expansion that is often associated with habitat alteration (e.g., *Rana berlandieri*, *A. tigrinum*, *Bufo woodhousii* [Lannoo forthcoming]). Adverse effects on native populations have been demonstrated in some of these cases by virtue of hybridization, resulting in loss of the native genotype in the affected areas. For example, introduced "bait-bucket" *A. tigrinum* in California have interbred with native *Ambystoma californiense*, contributing to the threats faced by this species designated federally as "threatened" (Shaffer and Trenham forthcoming). Similar events have occurred with introduced and native populations of *A. tigrinum* in Arizona (Storfer et al. forthcoming).

Invasive crayfish from the eastern and central U.S. have become a concern for native amphibians primarily in the southwestern U.S. Crayfish (*Orconectes virilis*) now occur in many streams in Arizona where virtually all aquatic vegetation, macroinvertebrates, and the native frog *R. chiricahuensis* have been eliminated subsequent to crayfish invasion (Fernandez and Rosen 1996). In laboratory experiments, crayfish killed and consumed newly hatched embryos, tadpoles, and small and large frogs. Gamradt and Kats (1996) reported the lack of California newt (*Taricha torosa*) eggs, larvae, and adults in streams with crayfish (*Procambarus clarkii*) in southern California and observed the reestablishment of the newt population in one stream following the elimination of crayfish by flooding. Their field experiments demonstrate predation by crayfish on newt eggs and larvae and indicate sufficient aggression by crayfish toward adults to deter breeding (Gamradt and Kats 1996; Gamradt et al. 1997).

There is sufficient evidence to assert that invasive species are a significant factor in the population declines of a number of amphibian species. Nevertheless, uncertainty remains in many cases, and even where invasive species are strongly implicated, the relative contribution of the various invasive species is often not clear. Moreover, adverse effects documented for one species or area do not necessarily apply to other species, areas, or ecological circumstances. Such uncertainties can make it difficult for a management agency to give high priority to actions addressing the effects of invasive species on amphibians when there are numerous other environmental concerns confronting the agency. But to wait for definitive studies involving an often historical event seems irresponsible.

Mechanisms of adverse effects

As discussed above, an understanding of the mechanisms of interaction among an invasive species and a native amphibian can provide an explanation for adverse effects observed at the population level, and may provide insight about potential control methods. Mechanisms of interaction include direct effects such as predation, competition, and hybridization, and several possible indirect effects.

Predation is often the presumed mode of interaction between invasive species and native amphibians because many of the invasive species, such as trout and centrarchid fishes, are known predators of small aquatic vertebrates. Typically, the affected native amphibian lacks defense mechanisms against predation from larger vertebrates, such as unpalatable eggs or larvae (Kats et al. 1988). Predation in the field is rarely observed when an invasive species gains access to a new site, but it is occasionally documented by dietary analysis (e.g., Gillespie and Hero 1999; Goodsell and Kats 1999; Crayon forthcoming). More frequently, the potential for significant predation in the field is evaluated by laboratory or mesocosm experiments or field trials, particularly for the smaller potential predators such as mosquitofish (e.g., Sexton and Phillips 1986; Gillespie and Hero 1999). Such studies have shown that the relatively small, but widely distributed, mosquitofish can feed on larvae and eggs of a variety of amphibian species (Grubb 1972; Gamradt and Kats 1996; Gillespie and Hero 1999; Goodsell and Kats 1999). Most studies address predation on larvae or eggs rather than recently metamorphosed individuals or adults. In some cases, aggressive behavior alone is sufficient to affect a native amphibian adversely. For example, aggressive behavior by crayfish was sufficient to exclude adult California newts from a site (Gamradt et al. 1997), and the presence of either smallmouth bass (*Micropterus dolomieu*) or adult bullfrogs in enclosures caused tadpoles of the red-legged frog (*Rana aurora*) to change microhabitat use (Kiesecker and Blaustein 1998). Hopey and Petranka (1994) provided evidence using artificial ponds that adult wood frogs (*Rana sylvatica*) avoid ovipositing in ponds containing predatory sunfish. They suggested that rapid shifts in species distributions among local ponds following the introduction of fishes may reflect adult avoidance behavior more so than direct predation of fish on eggs or larvae.

Competition for resources may be an important mechanism of negative effects between invasive and native species. In an extensive study of interactions among the invasive American bullfrog and two native anurans, the foothill yellow-legged frog (*Rana boylii*) and Pacific treefrog (*P. regilla*), experimental evidence indicated that bullfrog tadpoles can have strong effects on native tadpoles, whereas native tadpoles have weak effects on bullfrog tadpoles (Kupferberg 1997). For example, ambient densities of bullfrog larvae in field enclosures caused a substantial reduction in survivorship and mass at metamorphosis for *R. boylii* and a significant reduction in size at metamorphosis for *P. regilla*. Competition appeared to be mediated by algal resources rather than behavioral or chemical interference (Kupferberg 1997). In other studies with larval red-legged frogs (*R. aurora*), time to metamorphosis increased and mass at metamorphosis decreased when *R. aurora* tadpoles were exposed to larval bullfrogs (Kiesecker and Blaustein 1998; Kiesecker et al. 2001). Kiesecker et al. (2001) further demonstrated in mesocosms that the negative effects of bullfrog tadpoles on *R. aurora* tadpoles depended on clumping of the food resource; such clumping appears to occur commonly in the field due to habitat modification that results in conversion of shallow, ephemeral wetlands to smaller permanent ponds. These negative findings for tadpole interactions indicate that the control of juvenile or adult bullfrogs may not be sufficient to protect native amphibians from adverse effects of this invasive species. As a form of interference competition, the toxic eggs and larvae of the invasive marine toad may diminish the populations of some native amphibians in

Australia. Extensive mortality has been observed among several species of native tadpoles as a result of feeding on marine toad tadpoles and eggs (Hero and Stoneham forthcoming).

Hybridization between native amphibians and closely related taxa introduced from elsewhere has been demonstrated for California tiger salamander populations in California and the tiger salamander populations in Arizona (Shaffer and Trenham forthcoming; Storfer et al. forthcoming). Such hybridization potentially can result in loss of the native genotype over a large area, which greatly complicates efforts to protect and restore populations with the native genotype of the threatened California tiger salamander (Shaffer and Trenham forthcoming) and Sonora tiger salamander (Storfer et al. forthcoming).

Several indirect effects have been demonstrated or postulated between invasive species and native amphibians. Kiesecker et al. (2001) provided evidence suggesting that hatchery-reared fishes may serve as a vector for a pathogenic oomycete, *Saprolegnia ferax*, an organism associated with embryonic mortality of amphibians in the Cascade Mountains of Oregon (Blaustein et al. 1994). Increased sensitivity to other stressors may also be a consequence of the presence of invasive species. For example, sensitivity to a common pesticide, carbaryl, was increased for larvae of the gray treefrog (*Hyla versicolor*) when exposed to predatory cues (Relyea and Mills 2001). Habitat structure has been dramatically changed by some invasive animal species. In some streams in Arizona, crayfish have removed essentially all aquatic vegetation that otherwise would be used for cover by larval and adult anurans (Fernandez and Rosen 1996). Also, in forest ecosystems of the eastern U.S., populations of at least three species of salamanders appeared to have been adversely affected as a result of forest defoliation by the introduced gypsy moth and hemlock wooly adelgid (Bradford forthcoming).

The challenge to develop tools for mitigation and restoration

The task of reversing the impacts of invasive species on amphibians is fraught with difficulties. Three principal concerns are the prevention of further introduction of nonnative species, eradication of existing populations of invasive species, and reestablishment of amphibian populations following eradication.

In areas to be restored, programs to introduce invasive species (e.g., stocking of hatchery-reared game fish and mosquitofish) should be terminated. Such termination is a prerequisite to further restoration efforts, such as eradication or reduction of numbers of invasive animals. In some cases, populations of the invasive species will go extinct after stocking is terminated due to lack of suitable conditions for spawning (Knapp 1996). The importation of invasive species used as bait should be terminated in problem areas. For example, California has banned the importation of tiger salamanders as fish bait, largely in response to hybridization with native tiger salamanders. Efforts should be increased through regulation and education to prevent the commercial trade in exotic species that are potential threats (e.g., *X. laevis*) and to minimize the potential for accidental or deliberate release of animals held as pets or used in educational or research institutions.

The greatest challenge in most cases will be the eradication of invasive populations and the prevention of re-invasion. This is often very difficult because conditions suitable for the invasive and native species are typically quite similar. Moreover, restoration should not be restricted to isolated sites, but should be done in a metapopulation context (Semlitsch 2000), which further complicates the task. Some of the potential tools for eradication of invasive species include pond draining, manipulation of hydroperiod, seines, gill nets, traps, fencing, electroshocking, poisons, shooting, and the introduction of nonreproducing or removable predatory fish. Salmonid populations have been successfully eliminated using gill nets or piscicides in smaller mountain lakes where natural cascades prevent recolonization from downstream waters (Knapp and Matthews 1998; Parker et al. 2001; V. Vredenberg, University of California Berkley, Berkley, California, USA, unpublished data; R.A. Knapp, University of California Santa Barbara, Santa Barbara, California, USA, personal communication). Knapp and Matthews (1998) estimated that gill netting would be a viable eradication method in 15% to 20% of the high mountain lakes in the Sierra Nevada. Efforts with invasive amphibians have been less promising, although *X. laevis* was successfully eradicated by poisoning in one discrete body of water in California (Crayon forthcoming). Bullfrogs also have been eradicated from areas in southeastern Arizona, primarily by a combination of fencing and draining ponds (C.R. Schwalbe et al., University of Arizona, Tucson, Arizona, USA, personal communication). Prevention of re-invasion may require additional efforts. For example, habitat modification or hydrological management may be necessary to restore natural processes, such as more frequent pond drying (Adams 1999; Semlitsch 2000).

Following the eradication of invasive populations, the native amphibian population may expand or become reestablished if an extant population is already present or located nearby. If this is not the case, however, translocation of individuals from distant populations would be warranted. An initial concern with translocation is the selection of a source population or populations, weighing such factors as probable genetic similarity to the former population, genetic diversity among the translocated individuals, and impact to the source population (or populations). Moreover, techniques for successful translocation and population reestablishment are poorly known, and will likely differ considerably among taxa (Snyder et al. 1996; World Conservation Union 1998). For example, there is no general consensus about either the life stage that is likely to be most effective in achieving reestablishment nor the number of individuals necessary. Despite these concerns, some translocations have been successful. For example, spawn from multiple clutches of the natterjack toad (*Bufo calamita*) has been translocated to newly restored habitats in Britain, resulting in the successful establishment of a breeding population in most sites (Denton et al. 1997). For relatively rare species, "head-starting" (i.e., rearing of eggs or tadpoles through metamorphic stages) or captive breeding may be required. These techniques present additional concerns, such as reduced genetic diversity and disease transmission (Snyder et al. 1996; World Conservation Union 1998).

Research needs focused on invasive species

Research is needed to address several biological and management issues, including the following discussed below.

Cause–effect relationships

In most cases where an invasive species is suspected as a causal agent in amphibian population declines, solid evidence for cause and effect is lacking. Such evidence is necessary to justify corrective actions. In general, well-controlled field experiments in which invasive species are added to or removed from a system provide convincing evidence for the effects of the invasive species on the native amphibian, but such experiments may be difficult to conduct and replicate. An alternative approach, which must be done at many sites, is to conduct a multivariate analysis of distribution or abundance of the native species that includes parameters for various environmental factors as well as the invasive species.

Eradication and control techniques

In general, efforts to eradicate and control populations of invasive animals affecting native amphibians have only recently begun. Further investigations are needed to improve the efficacy of the various techniques, and to facilitate identification of the environmental conditions suitable for eradication or control.

Translocation and population reestablishment

The restoration of sites in many circumstances will require the translocation of amphibians from elsewhere. Techniques to accomplish this, however, are in their infancy. Moreover, the protocols suitable for one species or situation may not be suitable for another. Some other concerns for translocation include genetic similarity between the donor population and the population being replaced, genetic diversity of the new population, and disease transmission. These concerns regarding translocation are in addition to the broader concerns of determining how much habitat and how many populations need to be restored to ensure long-term persistence.

Interactions between invasive species and other factors, such as disease transmission

The suggestion that invasive species may be vectors for pathogens is alarming. Hatchery-reared fish, which possibly could be vectors of *Saprolegnia ferax,* are introduced to thousands of sites in various parts of the world on a regular basis. Moreover, the chytrid fungus, *B. dendrobatidis*, has devastated many amphibian populations (see "Chytridiomycosis," p 155) and possibly could be transmitted from invasive amphibians to natives. For example, the invasive American bullfrog appears to be relatively tolerant to the pathogen (P. Daszak, Strieby, and Porter, Consortium for Conservation Medicine, Palisades, New York, USA, unpublished data) and, thus, could potentially serve as a persistent carrier throughout western North America and elsewhere.

Mechanisms of interaction between invasive and native species

An understanding of the modes of interaction between the invasive and the native species may facilitate the development of suitable mitigation programs. For example, it can be useful to know whether the interaction operates by predation versus competition, what life stages are involved, and what environmental and temporal conditions facilitate or inhibit the negative interaction.

Role of Biotic Factors in Amphibian Deformities

Amphibian deformities have been reported in the scientific literature for at least three centuries. Deformities have been described throughout the world, at frequent intervals, and in many species of anurans and salamanders (Ouellet 2000). Most of the recorded deformities and malformations involve missing digits (ectrodactyly), missing limbs (ectromelia), extra digits (polydactyly), or extra limbs (polymely). Deformities may be due to either traumatic injury or true developmental abnormalities, and caution must be used in their diagnosis. Limb deformities, traumatic injuries, and other developmental abnormalities occur normally at frequencies of 0% to 5% in wild amphibian populations (Rostand 1949; Dubois 1979; Borkin and Pikulik 1986; Luis and Báez 1987; Meyer-Rochow and Asashima 1988; Read and Tyler 1994). However, over the last decade, an increasing number of amphibian populations exhibit abnormally high frequencies (>5%) of deformities (Sessions and Ruth 1990; Veith and Viertel 1993; Flax and Borkin 1997; Ouellet et al. 1997; Johnson et al. 1999; Johnson, Lunde, Ritchie, et al. 2001). This apparent recent increase in amphibian deformities has attracted international attention.

Multiple causes and hypotheses to explain amphibian deformities

Many biotic and abiotic factors have been proposed as causative agents of amphibian limb deformities: abnormal regeneration after injury (e.g., following attempted predation), effects of agricultural pesticides and fertilizers, chemical composition of the water (pH), coexistence with certain fishes (*Anguilla* sp., *Tinca* sp.), diseases (aflatoxins), elevated tadpole densities, extreme temperatures (30 °C), hereditary mechanisms (genetic mutations, hybridization), nutritional deficiencies, osteolathyrogenic agents (synthetic lathyrogenic compounds), presence of parasitic trematode cysts, radioactive pollution, retinoids (vitamin A and derivatives, synthetic terpenoids), teratogenic viruses, trace metals, UV-B radiation, and various xenobiotic chemicals (including endocrine disruptors, pharmaceutical products) (see review by Ouellet 2000). Nevertheless, trauma, parasitic trematode infection, and xenobiotic contaminants (perhaps affecting retinoid receptor pathways), along with a synergistic action of UV-B radiation, emerge as the leading hypotheses. No single cause will likely explain high frequencies of amphibian deformities in the wild (Veith and Viertel 1993; Ouellet et al. 1997; Gardiner and Hoppe 1999; Meteyer et al. 2000; Johnson, Lunde, Ritchie, et al. 2001; Johnson, Lunde, Haight, et al. 2001; Gray et al. 2002). The causes may be local or more regional in scale and are probably the result of a complex interaction of multiple factors. The following discussion will be limited to an exploration of the relationship of a biotic factor, trematode parasitism, to the production of amphibian malformations.

Trematode parasites and amphibian deformities

Among the biotic factors proposed to explain amphibian limb deformities, there is now compelling evidence that trematode parasites (flukes) are playing a major role in producing malformations at some localities. Parasites have long been suspected as a potential cause of limb duplications (Woïtkewitch 1961), but direct evidence of this has been presented only recently. Sessions and Ruth (1990) studied populations of Pacific treefrogs (*P. regilla*) and long-toed salamanders (*A. macrodactylum*) with high frequencies

of limb abnormalities, including supernumerary hindlimbs, from two adjacent ponds in California. Metacercarial cysts of digenetic trematodes were found preferentially located in the cloacal and developing hindlimb regions in larvae of both species. Certain species of these parasitic flatworms use amphibians as a second intermediate host in a complex life cycle in which the definitive host is usually a vertebrate (bird, fish, mammal, or reptile) and pond snails are the first intermediate host (Schell 1985; Esch and Fernandez 1994). Generally, the cercariae produced and released from the snail host will swim freely and penetrate the amphibians through the skin or cloaca to encyst as metacercariae. Sessions and Ruth (1990) hypothesized that metacercarial cysts of trematodes can interfere mechanically with normal limb development and regeneration by disrupting positional relationships between cells in the developing limb. This interference and subsequent disruption can lead to extra limbs in these animals. Experimentally, Sessions and Ruth (1990) induced duplicated distal limb structures by implanting inert resin beads of the same size as the metacercarial cysts in the developing limb buds of laboratory-raised African clawed frogs (*X. laevis*) and Mexican axolotls (*Ambystoma mexicanum*). In fact, extra hindlimbs with mirror-image duplications in the anteroposterior axis have been proposed as a characteristic feature of trematode infection of amphibians (Sessions and Ruth 1990; Sessions et al. 1999). Three types of digit patterns may be observed in the anteroposterior axis: posterior mirror-image duplication, anterior mirror-image duplication, and mirror-image triplication (Sessions et al. 1999).

In another study, high frequencies of hindlimb abnormalities, including cases of polymely, ectromelia, and cutaneous fusion, were induced in metamorphosing *P. regilla* exposed as tadpoles to living cercariae of *Ribeiroia ondatrae* (Trematoda: Psilostomidae) (Johnson et al. 1999). Based on field observations in California, experimental treatments were performed using exposures to realistic concentrations of cercariae and produced the same range and frequency of hindlimb malformations as observed in field-collected animals. An increase in *Ribeiroia* density in the exposures caused an increase in abnormality frequency and a decline in tadpole survivorship. Neither limb malformations nor an increase in mortality were observed in *P. regilla* exposed to the cercariae of a second species of trematode (*Alaria mustelae*) (Johnson et al. 1999). Both *Ribeiroia* and *Alaria* cercariae were isolated from naturally infected aquatic snails (*Planorbella tenuis*) collected in the area. In a second experiment, high frequencies of limb malformations and mortality were similarly induced in western toads (*B. boreas*) exposed to *R. ondatrae* cercariae obtained from infected planorbid snails from California (Johnson, Lunde, Haight, et al. 2001). While *P. regilla* malformations were dominated by hindlimb polymely (Johnson et al. 1999), cutaneous fusion predominated among *B. boreas* abnormalities. Differences in malformation types and their relative frequencies indicate that amphibian species may respond differently to *R. ondatrae* infection (Johnson, Lunde, Haight, et al. 2001). These two experiments fulfilled Koch's postulates by demonstrating that *R. ondatrae* can cause limb abnormalities in at least two amphibian species from specific localities of the western U.S. More recently, extensive field surveys have shown that *R. ondatrae* infection is actually linked to amphibian malformations in many other sites in the western U.S. (Johnson et al. 2002).

Implications of deformities in amphibian population declines

Amphibian deformities are maladaptive and affect survivorship by interfering with swimming, terrestrial movement, food acquisition, and predator avoidance. They are likely to affect population recruitment at local and regional scales. Few deformed juveniles survive to adulthood, based on low frequencies of deformities recorded with adults (Sessions and Ruth 1990; Veith and Viertel 1993; Ouellet et al. 1997; Johnson et al. 1999; Johnson, Lunde, Ritchie, et al. 2001). Deformed anurans might be particularly vulnerable to predation at metamorphosis (Arnold and Wassersug 1978). However, the overall influence on local populations of an increased incidence of amphibian deformities is difficult to ascertain. Annual variability in deformity rates could lessen the impact on local populations, and a population-level impact might be possible only where rates are consistently high from year to year. Otherwise, natural annual variability in amphibian recruitment rates might mask any effects of the deformities. Earlier studies provide little guidance; and at least in one, where limb malformations were abundant in the 1950s and 1960s, anuran survivorship was already a matter of concern (Rostand 1955, 1958, 1971; Rostand and Darré 1968). Severe cases of malformations were always lethal at the metamorphic stage because of injuries and hemorrhages attributed to mechanical interferences of the abnormal hindlimbs. Even today, an increased incidence of deformities in any area appears to be site specific, and there is no real evidence of a common global phenomenon. Mass deformities, however, could exacerbate amphibian declines in already threatened populations. Consideration should also be given to the actual causes of such deformities. For example, increased parasitic trematode infection might by itself represent a source of mortality at the larval stage in some amphibian populations (Etges 1961; Fried et al. 1997; Johnson et al. 1999; Johnson, Lunde, Haight 2001). Since several different factors may cause limb abnormalities, population level studies will be required to determine how much mass deformities contribute to amphibian declines at local, regional, or global scales.

Research needs into the biotic causes of amphibian deformities

More research is needed on the real significance of digenetic trematode infection in different species of amphibians. Little is known on the geographic distribution, prevalence, and certain aspects of the life cycle of the genus *Ribeiroia* (Beaver 1939; Riggin 1956; Basch and Sturrock 1969). The natural occurrence of *Ribeiroia* sp. in potential amphibian hosts is virtually unknown. Can *R. ondatrae* cause similar malformations in other amphibian species? Are other trematode species implicated in amphibian deformities? What are the precise developmental and cellular mechanisms involved?

Clearing and differential staining techniques for bone and cartilage (Wassersug 1976; Hanken and Wassersug 1981) can be used on freshly dead or appropriately preserved specimens to characterize deformities and search for metacercarial cysts in relation to limb structures, normal or deformed. Trematode cysts can also be identified following a careful dissection of malformed amphibians (Johnson et al. 1999). It should be emphasized that the presence of cysts does not necessarily demonstrate a causal relationship. Many trematode species are known to infect larval and adult amphibians (Etges 1961; Cook 1978; Shields 1987; Martin and Conn 1990; Fried et al. 1997; Marcogliese

et al. 2000). Furthermore, the prevalence of deformed amphibians and the occurrence of *Ribeiroia* sp. are expected to vary from region to region (Gillilland and Muzzall 2002). The experimental induction of limb malformations using living trematode cercariae remains to be performed in different amphibian species from distinct geographic areas.

Fluctuations in aquatic snail populations, snail–tadpole (Holomuzki and Hemphill 1996), snail–trematode, and trematode–trematode interactions also warrant further study. Host animals and their parasites usually exist in relative equilibrium in most environments. Trematode communities in molluscan intermediate hosts are highly structured, are very dynamic in character, and reflect long periods of coevolution (Esch and Fernandez 1994). Are trematodes affecting the amphibian intermediate host to increase transmission to the potential definitive hosts? Could the production of malformations in the amphibian host therefore be explained by an evolutionary relationship between amphibian and trematode, which increases the likelihood of predation of the intermediate host? Can breeding amphibians protect offspring from potential trematode infection by preferentially ovipositing in areas relatively free of parasites (Kiesecker and Skelly 2000)? Anthropogenic habitat degradation and fragmentation, introduced species, and poor water quality may influence snail dynamics, trematode abundance and interactions, and host behavior. Environmental deterioration may also compromise the immune system of amphibian hosts and alter their susceptibility to parasite infection. Kiesecker (2002) has linked increased trematode infection, and increased limb deformities, to pesticide exposure in wood frogs (*R. sylvatica*). All these questions call for further research on trematode parasitism, environmental factors, and their effects on the health of amphibian populations.

Acknowledgments

Preparation of this review and the research generating unpublished data cited in this review were supported by an Integrated Research Challenges in Environmental Biology (IRCEB) grant IBN-9977063 from the National Science Foundation. We thank Roland Knapp and Donald Nichols for providing comments on this chapter. Cecil Schwalbe and Philip Rosen are thanked for supplying information and documents pertaining to invasive species. The U.S. Environmental Protection Agency (USEPA), through its Office of Research and Development, contributed to the analyses described herein. This chapter has been subjected to the USEPA's review and has been approved for publication.

References

Adams MJ. 1999. Correlated factors in amphibian decline: Exotic species and habitat change in western Washington. *J Wildl Manage* 63:1162–1171.

Ahne W, Bearzotti M, Bremont M, Essbauer S. 1998. Comparisons of European systemic piscine and amphibian iridoviruses with Epizootic Haematopoietic Necrosis Virus and Frog Virus 3. *J Vet Med B* 45:373–383.

Alexander MA, Eischeid JK. 2001. Climate variability in regions of amphibian declines. *Conserv Biol* 15:930–942.

Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory mycology, 4th edition. New York, NY, USA: John Wiley & Sons. 868 p.

Alford RA, Richards SJ. 1999. Global amphibian declines: a problem in applied ecology. *Annu Rev Ecol Syst* 30:133–165.

Anderson RM, May RM. 1991. Infectious diseases of humans: dynamics and control. Oxford, UK: Oxford University Press. 757 p.

Arnold SJ, Wassersug RJ. 1978. Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. *Ecology* 59:1014–1022.

Bahls PF. 1992. The status of fish populations and management of high mountain lakes in the western United States. *Northwest Sci* 66:183–193.

Barr DJS. 1980. An outline for the reclassification of the Chytridiales, and for a new order, the Spizellomycetales. *Can J Bot* 58: 2380–2394.

Barr DJS. 2000. Chytridiomycota. In: McLaughlin DJ, McLaughlin EG, Lemke PA, editors. The mycota VII: Systematics and evolution part A. Berlin, DE: Springer-Verlag. p 93–112.

Basch PF, Sturrock RF. 1969. Life history of *Ribeiroia marini* (Faust and Hoffman, 1934) comb. n. (Trematoda: Cathaemasiidae). *J Parasitol* 55:1180–1184.

Beaver PC. 1939. The morphology and life history of *Psilostomum ondatrae* Price, 1931 (Trematoda: Psilostomidae). *J Parasitol* 25:383–393.

Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, Slocombe R, Ragan MA, Hyatt AD, McDonald KR, Hines HB, Lips KR, Marantelli G, Parkes H. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc Natl Acad Sci USA* 95:9031–9036.

Berger L, Speare R, Hyatt A. 2000. Chytrid fungi and amphibian declines: Overview, implications and future directions. In: Campbell A, editor. Declines and disappearances of Australian frogs. Canberra, AU: Environment Australia. p 21–31.

Berna HJ. 1990. Ecology and life history of the tiger salamander, *Ambystoma tigrinum nebulosum* Hallowell, on the Kaibab Plateau. Tempe, AZ, USA: Arizona State University Deparment of Biology. 121 p.

Blaustein AR, Hokit DG, O'Hara RK, Holt RA. 1994. Pathogenic fungus contributes to amphibian losses in the Pacific Northwest. *Biol Conserv* 67:251–254.

Bollinger TK, Mao J, Schock D, Brigham RM, Chinchar VG. 1999. Pathology, isolation and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. *J Wildl Dis* 35:413–429.

Borkin LJ, Pikulik MM. 1986. The occurrence of polymely and polydactyly in natural populations of anurans of the USSR. *Amphib Rept* 7:205–216.

Bosch J, Martínez-Solano I. García-París M. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad *(Alytes obstetricans)* in protected areas of central Spain. *Biol Conserv* 97:331–337.

Bradford DF. 1983. Winterkill, oxygen relations, and energy metabolism of a submerged dormant amphibian, *Rana muscosa*. *Ecology* 64:1171–1183.

Bradford DF. 1989. Allotopic distribution of native frogs and introduced fishes in high Sierra Nevada lakes of California: implication of the negative effects of fish introductions. *Copeia* 1989:775–778.

Bradford DF. 1991. Mass mortality and extinction in a high-elevation population of *Rana muscosa*. *J Herpetol* 25 174–177.

Bradford DF. Epilogue: Factors implicated in amphibian population declines in the United States. In: Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Bradford DF, Cooper SD, Jenkins TM Jr, Kratz K. Sarnelle O, Brown AD. 1998. Influences of natural acidity and introduced fish on faunal assemblages in California alpine lakes. *Can J Fish Aquat Sci* 55:2478–2491.

Bradford DF, Graber DM, Tabatabai F. 1994. Population declines of the native frog, *Rana muscosa*, in Sequoia and Kings Canyon National Parks, California. *Southwestern Nat* 39:323–327.

Bradford DF, Swanson C, Gordon MS. 1992. Effects of low pH and aluminum on two declining species of amphibians in the Sierra Nevada, California. *J Herpetol* 26:369–377.

Bradford DF, Tabatabai F, Graber DM. 1993. Isolation of remaining populations of the native frog, *Rana muscosa*, by introduced fish in Sequoia and Kings Canyon National Parks, California. *Conserv Biol* 7:882–888.

Bradley GA, Rosen PC, Sredl MJ, Jones TR, Longcore JE. 2002. Chytridiomycosis in native Arizona frogs *(Rana yavapaiensis, Rana chiricahuensis,* and *Hyla arenicolor). J Wildlife Dis* 38:206–212.

Braña F, Frechilla L, Orizaola G. 1996. Effect of introduced fish on amphibian assemblages in mountain lakes of northern Spain. *Herpetol J* 6:145–148.

Brönmark C, Edenhamn P. 1994. Does the presence of fish affect the distribution of tree frogs (*Hyla arborea*)? *Conserv Biol* 8:841–845.

Brunner JL, Schock DM, Davidson EW, Collins JP. 2003. Intraspecific reservoirs: Complex life history and the persistence of a lethal ranavirus. *Ecology*. Forthcoming.

Burrowes PA, Joglar RL. 2002. The status of the Puerto Rican amphibians. In: Wilkinson JW, editor. DAPTF Collected geographical working group reports 2001. DAPTF/IUCN, Publishers.

Bury RB, Wheelan JA. 1984. Ecology and management of the bullfrog. U.S. Fish and Wildlife Service Resource Publication 155.

Carey C. 1993. Hypothesis concerning the disappearance of boreal toads from the mountains of Colorado. *Conserv Biol* 7:355–362.

Carey C. 2000. Infectious disease and worldwide declines of amphibian populations, with comments on emerging diseases in coral reef organisms and in humans. *Environ Health Perspect* 108, Supplement 1:143–150.

Carey C, Cohen N, Rollins-Smith L. 1999. Amphibian declines: an immunological perspective. *Devel Comp Immunol* 23:459–472.

Casper GS, Hendricks R. *Rana catesbeiana* Shaw, 1802; American bullfrog. In: Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Chinchar VG. 2002. Ranaviruses (Family Iridoviridae): Emerging cold-blooded killers. *Arch Virol* 147(3):447–470.

Clark HF, Brennan JC, Zeigel RF, Karzon DT. 1968. Isolation and characterization of viruses from the kidneys of *Rana pipiens* with renal adenocarcinoma before and after passage in the red eft (*Triturus viridescens*). *J Virol* 2:629–640.

Clark HF, Gray C, Fabian F, Zeigel R, Karzon DT. 1969. Comparative studies of amphibian cytoplasmic virus strains isolated from the leopard frog, bullfrog, and newt. In: Mizell M, editor. Biology of amphibian tumors. New York, NY, USA: Springer-Verlag. p 310–326.

Collins JP. 1981. Distribution, habitats and life history variation in the tiger salamander, *Ambystoma tigrinum*, in East-central and Southeast Arizona. *Copeia* 1981: 666–675.

Collins, JP, Jones TR, Berna HJ. 1988. Conserving genetically-distinctive populations: The case of the Huachuca tiger salamander (*Ambystoma tigrinum stebbinsi* Lowe). In: Szaro RC, Severson KE, Patton DR, editors. Management of amphibians, reptiles, and small mammals in North America. Fort Collins, CO, USA: U.S. Department of Agriculture Forest Service, Rocky Mountain Forest and Range Experiment Station. GTR-RM-166. p 45–53

Collins JP, Zerba KE, Sredl MJ. 1993. Shaping intraspecific variation: development, ecology and the evolution of morphology and life history variation in tiger salamanders. *Genetica* 89:167–183.

Cook TW. 1978. The migration of *Fibricola cratera* (Trematoda: Diplostomatidae) in metamorphosing *Rana pipiens*. *J Parasitol* 64:938–939.

Corn PS. 2000. Amphibian declines: Review of some current hypotheses. In: Sparling DW, Linder G, Bishop CA, editors. Ecotoxicology of amphibians and reptiles. Pensacola, FL, USA: SETAC. p 663–696.

Corn PS, Jennings ML, Muths E. 1997. Survey and assessment of amphibian populations in Rocky Mountain National Park. *Northwestern Nat* 78:34–55.

Cory L. 1963. Effects of introduced trout on the evolution of native frogs in the high Sierra Nevada mountains. *Proc XVI Inter Congr Zool* 1963(2):172.

Crawshaw GJ 2000. Diseases and pathology of amphibians and reptiles. In: Sparling DW, Linder G, Bishop CA, editors. Ecotoxicology of amphibians and reptiles. Pensacola, FL, USA: SETAC. p 199–231.

Crayon JJ. *Xenopus laevis* (Daudin, 1802); African clawed frog. In: Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Cullen BR, Owens L, Whittington RJ. 1995. Experimental infection of Australian anurans (*Limnodynastes terraereginae* and *Litoria latopalmata*) with Bohle iridovirus. *Dis Aquat Org* 23:83–92.

Cunningham AA, Langton TES, Bennet PM, Drury SEN, Gough RE, Kirkwood JK. 1993. Unusual mortality associated with poxvirus-like particles in frogs (*Rana temporaria*). *Vet Rec* 133: 141–142.

Cunningham AA, Langton TES, Bennet PM, Lewin JF, Drury SEN, Gough RE, MacGregor SK. 1995. Investigations into unusual mortalities of the common frog (*Rana temporaria*) in Britain. In: Zwart P, Matz G, editors. Proceedings of the fifth international colloquium on the pathology of reptiles and amphibians. 1995 Mar 31–Apr 2; Alphen Aan Den Rijn, NL. p 19–27.

Cunningham AA, Langton TES, Bennet PM, Lewin JF, Drury SEN, Gough RE, MacGregor SK. 1996. Pathological and microbiological findings from incidents of unusual mortality of the common frog (*Rana temporaria*). *Phil Trans R Soc Lond B Biol Sci* 351:1539–1557.

[DAPTF] Declining Amphibians Population Task Force. 2001. The DAPTF Fieldwork Code of Practice. Available from: John Wilkinson, Biology Department, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK.

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, Speare R. 1999. Emerging infectious diseases and amphibian population declines. *Emerg Infect Dis* 5:735–748.

Davidson C, Shaffer HB, Jennings MR. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conserv Biol* 16: 1588–1601.

Davidson EW, Pessier AP, Longcore JE, Parris M, Jancovich J, Brunner J, Schock D, Collins JP. 2000. Chytridiomycosis in Arizona (USA) tiger salamanders. In: Getting the jump! On amphibian diseases: proceedings; 2000 Aug 26–27; Cairnes, Queensland, AU.

Denton JS, Hitchings SP, Beebee TJC, Gent A. 1997. A recovery program for the natterjack toad *(Bufo calamita)* in Britain. *Conserv Biol* 11:1329–1338.

Desroches JF, Ouellet M. 2001. Characteristics, movements, and health of a western chorus frog (*Pseudacris triseriata*) population at a breeding pond in southwestern Québec. Canadian amphibian and reptile conservation network (CARCNET) annual meeting; 2001 Oct 22–25; Cornwall, Prince Edward Island, CA: CARCNET.

Docherty D, Chinchar VG, Meteyer C, Brannian R, Hansen W, Wang J, Mao J. 1999. A diagnostic evaluation of three salamander mortality events associated with an iridovirus and subsequent genomic comparison of the virus isolates obtained. 48th Annual Wildlife Disease Association (WDA) conference; 1999 Aug 8–12; Athens, GA, USA: WDA.

Drost CA, Fellers GM. 1996. Collapse of a regional frog fauna in the Yosemite area of the California Sierra Nevada. *Conserv Biol* 10:414–425.

Drury SEN, Gough RE, Cunningham AA. 1995. Isolation of an iridovirus-like agent from common frogs (*Rana temporaria*). *Vet Rec* 137:72–73.

Dubois A. 1979. Anomalies and mutations in natural populations of the *Rana "esculenta"* complex (Amphibia, Anura). *Mitt Zool Mus Berlin* 55:59–87.

Esch GW, Fernandez JC. 1994. Snail-trematode interactions and parasite community dynamics in aquatic systems: a review. *Am Midl Nat* 131:209–237.

Etges FJ. 1961. Contributions to the life history of the brain fluke of newts and fish, *Diplostomulum scheuringi* Hughes, 1929 (Trematoda: Diplostomatidae). *J Parasitol* 47:453–458.

Fellers GM, Green DE, Longcore JE 2001. Oral chytridiomycosis in the mountain yellow-legged frog *(Rana muscosa). Copeia* 2001:945–953.

Fernandez PJ, Rosen PC. 1996. Effects of the introduced crayfish *Orconectes virilis* on native aquatic herpatofauna in Arizona. Final report. Phoenix, AZ, USA: Arizona Department of Game and Fish. IIPAM Project nr. 194054.

Fijan N, Matasin Z, Petrinec Z, Valpotic I, Zwillenberg LO. 1991. Isolation of an iridovirus-like agent from green frogs (*Rana esculenta* L.). *Veterinarski Arhiv* 61:151–158.

Fisher RN, Shaffer HB. 1996. The decline of amphibians in California's Great Central Valley. *Conserv Biol* 10:1387–1397.

Flax NL, Borkin LJ. 1997. High incidence of abnormalities in anurans in contaminated industrial areas (eastern Ukraine). In: Böhme W, Bischoff W, Ziegler T, editors. Herpetologia bonnensis. Bonn, DE: Societas Europaea Herpetologica (SEH). p 119–123.

Fried B, Pane PL, Reddy A. 1997. Experimental infection of *Rana pipiens* tadpoles with *Echinostoma trivolvis* cercariae. *Parasitol Res* 83:666–669.

Frank SA. 1997. Spatial process in host-parasite genetics. In: Hanski IA , Gilpin ME, editors. Metapopulation biology: ecology, genetics and evolution. New York, NY, USA: Academic Press. p 325–358.

Froglog 48. 2001. Froglog Shorts: Chytridiomycosis threatens native frogs in New Zealand. *Froglog* 48:9. Available from: http://www2.open.ac.uk/biology/froglog/FROGLOG-48-9.html. Accessed 2003 May.

Funk WC, Dunlap WW. 1999. Colonization of high-elevation lakes by long-toed salamanders (*Ambystoma macrodactylum*) after the extinction of introduced trout populations. *Can J Zool* 77:1759–1767.

Gamradt SC, Kats LB. 1996. Effect of introduced crayfish and mosquitofish on California newts. *Conserv Biol* 10:1155–1162.

Gamradt SC, Kats LB, Anzalone CB. 1997. Aggression by nonnative crayfish deters breeding in California newts. *Conserv Biol* 11:793–796.

Gardiner DM, Hoppe DM. 1999. Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). *J Exp Zool* 284:207–216.

Gillespie GR. 2001. The role of introduced trout in the decline of the spotted tree frog *(Litoria spenceri)* in south-eastern Australia. *Biol Conserv* 100:187–198.

Gillespie GR, Hero JM. 1999. Potential impacts of introduced fish and fish translocations on Australian amphibians. In: Campbell A, editor. Declines and disappearances of Australian frogs. Canberra, AU: Environment Australia. p 131–144.

Gillilland MG, Muzzall PM. 2002. Amphibians, trematodes, and deformities: An overview from southern Michigan. *Comp Parasitol* 69:81–85.

Goodsell JA, Kats LB. 1999. Effect of introduced mosquitofish on Pacific treefrogs and the role of alternative prey. *Conserv Biol* 13:921–924.

Granoff A. 1969. Viruses of amphibia. *Curr Top Microbiol Immunol* 50:107–137.

Granoff A, Came PE, Breeze DC. 1966. Viruses and renal carcinoma of *Rana pipiens* I. the isolation and properties of virus from normal and tumor tissue. *Virology* 29:133–148.

Granoff A, Came PE, Rafferty KA. 1965. The isolation and properties of viruses from *Rana pipiens*: Their possible relationship to the renal adenocarcinoma of the leopard frog. *Ann NY Acad Sci* 126:237–255.

Gray HM, Ouellet M, Green DM, Rand AS. 2002. Traumatic injuries in two neurotropical frogs, *Dendrobates auratus* and *Physalaemus pustulosus*. *J Herpetol* 36:117–121.

Green DE 2001. Chytidiomycosis of amphibians: historic, epizootiological & pathological aspects [abstract]. In: 6th International Symposium on the Pathology of Reptiles and Amphibians; 2001 Apr 18–20; St. Paul MN, USA: University of Minnesota.

Green DE, Converse KA, Schrader AK. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996-2001. *Ann N Y Acad Sci* 969: 323–339.

Green DE, Sherman CK. 2001. Diagnostic histological findings in Yosemite toads *(Bufo canorus)* from a die-off in the 1970s. *J Herpetol* 35:92–103.

Grenfell B, Harwood J. 1997. (Meta)population dynamics of infectious diseases. *Trends Ecol Evol* 12:395–399.

Grinnell J, Storer TI. 1924. Animal life in the Yosemite. Berkeley, CA, USA: Univ Calif Press. 752 p.

Groff JM, Mughannam A, McDowell TS, Wong A, Dykstra MJ, Frye FL, Hedrick RP. 1991. An epizootic of cutaneous zygomycosis in cultured dwarf African clawed frogs (*Hymenochirus curtipes*) due to *Basidiobolus ranarum. J Med Vet Mycol* 29:215–223.

Grubb JC. 1972. Differential predation by *Gambusia affinis* on the eggs of seven species of anuran amphibians. *Amer Midl Nat* 88:102–108.

Hanken J, Wassersug R. 1981. The visible skeleton. *Funct Photogr* 16:22–26, 44.

Hanski IA. 1997. Metapopulation dynamics: from concepts and observations to predictive models. In: Hanski IA , Gilpin ME, editors. Metapopulation biology: ecology, genetics and evolution. New York: Academic Press. p 69–92.

Hanski IA, Gilpin M. 1991. Metapopulation dynamics: brief history and conceptual domain. *Biol J Linnean Soc* 42:3–16.

Hayes MP, Jennings MR. 1986. Decline of ranid frog species in western North America: are bullfrogs (*Rana catesbeiana*) responsible? *J Herpetol* 20:490–509.

Hecnar SJ, M'Closkey RT. 1997. The effects of predatory fish on amphibian species richness and distribution. *Biol Conserv* 79:123–131.

Hero JM, Stoneham M. *Bufo marinus* Linnaeus, 1758; marine toad (cane toad). In: Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Hird DW, Diesch SL, McKinnell RG, Gorham E, Martin FB, Kurtz SW, Dubrovolny C. 1981. *Aeromonas hydrophila* in wild-caught frogs and tadpoles *(Rana pipiens)* in Minnesota. *Lab An Sci* 31: 166–169.

Holomuzki JR, Hemphill N. 1996. Snail-tadpole interactions in streamside pools. *Am Midl Nat* 136: 315–327.

Hopey ME, Petranka JW. 1994. Restriction of wood frogs to fish-free habitats: how important is adult choice? *Copeia* 1994:1023–1025.

Hubbard GB. 1981. *Aeromonas hydrophilia* infection in *Xenopus laevis. Lab Anim Sci* 31: 297-300

Hyatt AD, Gould AR, Zupanovic Z, Cunningham AA, Hengstberger S, Whittington RJ, Kattenbelt J, Coupar BEH. 2000. Comparative studies of piscine and amphibian iridoviruses. *Arch Virol* 145:301–331.

Hyatt AD, Parkes H, Zupanovic Z. 1998. Identification, characterization and assessment of Venezuelan viruses for potential use as biological control agents against the cane toad (*Bufo marinus*) in Australia. Geelong, Victoria, AU: Commonwealth Scientific and Industrial Research Organisation (CSIRO).

James TY, Porter D, Leander CA, Vilgalys R, Longcore JE. 2000. Molecular phylogenetics of the Chytridiomycota supports the utility of ultrastructural data in chytrid systematics. *Can J Bot* 78:336–350.

Jancovich JK. 1999. *Ambystoma tigrinum* virus: identification, detection, transmission, characterization, and taxonomic position. Tempe, AZ, USA: Arizona State University Department of Biology. 59 p.

Jancovich JK, Davidson EW, Morado JF, Jacobs BL, Collins JP. 1997. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi Dis Aquat Org* 31:161–167.

Jancovich JK, Davidson EW, Seiler A, Jacobs BL, Collins JP. 2001. Transmission of the *Ambystoma tigrinum* virus to alternate hosts. *Dis Aquat Org* 46:159–163.

Jennings MR. 1996. Status of amphibians. In: Sierra Nevada ecosystem project: Final report to congress, volume 2. Davis, CA, USA: University of California Centers for Water and Wildlife Resources.

Jennings MR, Hayes MP. 1994. Amphibian and reptile species of special concern in California. Rancho Cordova, CA, USA: California Department of Fish and Game.

Johnson PTJ, Lunde KB, Haight RW, Bowerman J, Blaustein AR. 2001. *Ribeiroia ondatrae* (Trematoda: Digenea) infection induces severe limb malformations in western toads (*Bufo boreas*). *Can J Zool* 79:370–379.

Johnson PTJ, Lunde KB, Ritchie EG, Launer AE. 1999. The effect of trematode infection on amphibian limb development and survivorship. *Science* 284:802–804.

Johnson PTJ, Lunde KB, Ritchie EG, Reaser JK, Launer AE. 2001. Morphological abnormality patterns in a California amphibian community. *Herpetologica* 57:336–352.

Johnson PTJ, Lunde KB, Thurman EM, Ritchie EG, Wray SN, Sutherland DR, Kapfer JM, Frest TJ, Bowerman J, Blaustein AR. 2002. Parasite (*Ribeiroia ondratrae*) infection linked to amphibian malformations in the western United States. *Ecol Monogr* 72:151–168.

Karling JS. 1977. Chytridiomycetarum Iconographia. Monticello, NY, USA: Lubrecht and Cramer.

Kats L, Petranka JW, Sih A. 1988. Antipredator defenses and the persistence of amphibian larvae with fishes. *Ecology* 69:1865–1870.

Kattenbelt JA, Hyatt AD, Gould AR. 2000. Recovery of ranavirus dsDNA from formalin-fixed archival material. *Dis Aquat Org* 39:151–154.

Kiesecker JM. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? *Prod Natl Acad Sci USA* 99:9900–9904.

Kiesecker JM, Blaustein AR. 1998. Effects of introduced bullfrogs and smallmouth bass on microhabitat use, growth, and survival of native red-legged frogs (*Rana aurora*). *Conserv Biol* 12: 776–787.

Kiesecker JM, Blaustein AR, Miller CL. 2001. Transfer of a pathogen from fish to amphibians. *Conserv Biol* 15:1064–1070.

Kiesecker JM, Skelly DK. 2000. Choice of oviposition site by gray treefrogs: the role of potential parasitic infection. *Ecology* 81:2939–2943.

Knapp RA. 1996. Non-native trout in natural lakes of the Sierra Nevada: an analysis of their distribution and impacts on native aquatic biota. In: Sierra Nevada ecosystem project: Final report to Congress, volume 3. Davis, CA, USA: University of California, Centers for Water and Wildland Resources. p 363–407.

Knapp RA, Corn PS, Schindler DE. 2001. The introduction of nonnative fish into wilderness lakes: good intentions, conflicting mandates, and unintended consequences. *Ecosystems* 4:275–278.

Knapp RA, Matthews KR. 1998. Eradication of non-native fish by gill-netting from a small mountain lake in California. *Rest Ecol* 6:207–213.

Knapp RA, Matthews KR. 2000. Nonnative fish introductions and decline of the mountain yellow-legged frog (*Rana muscosa*) from within protected areas. *Conserv Biol* 14:428–438.

Knapp RA, Matthews KR, Sarnelle O. 2001. Resistance and resilience of alpine lake fauna to fish introductions. *Ecol Monogr* 71:401–421.

Kupferberg SJ. 1997. Bullfrog (*Rana catesbeiana*) invasion of a California river: the role of larval competition. *Ecology* 78:1736–1751.

Kuzmin S. 1994. The problem of declining amphibian populations in the Commonwealth of Independent States and adjacent territories. *Alytes* 12:123–134.

Lamirande EW, Nichols DK. 2002. Effects of host age on susceptibility to cutaneous Chytridiomycosis in blue- and yellow-poison dart frogs (*Dendrobates tinctorius*). In: McKinnell RG, Carlson DL, editors. 6th International Symposium on the Pathology of Reptiles and Amphibians; 2001 Apr 18–20; St. Paul MN, USA: University of Minnesota. p 3–16.

Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Lawler SP, Dritz D, Strange T, Holyoak M. 1999. Effects of introduced mosquitofish and bullfrogs on the threatened California red-legged frog. *Conserv Biol* 13:613–622.

Liss WJ, Larson GL, Deimling E, Ganio L, Gresswell R, Hoffman R, Kiss M, Lomnicky G, McIntire CD, Truitt R, Tyler T. 1995. Ecological effects of stocked trout in naturally fishless high mountain lakes, North Cascades National Parks Service Complex, Seattle, WA. Seattle, WA, USA: National Park Service, Pacific Northwest Region. Technical report NPS/PNROSU/NRTR-95-03

Lloyd AL, May RM. 1996. Spatial heterogeneity in epidemic models. *J Theoret Biol* 179:1–11.

Longcore JE, Pessier AP, Nichols DK. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219–227.

Luis R, Báez M. 1987. Anomalías morfológicas en los anfibios de las Islas Canarias (Amphibia, Anura). *Vieraea* 17:295–296.

Mao J, Green DE, Fellers G, Chinchar VG. 1999. Molecular characterization iridoviruses isolated from sympatric amphibians and fish. *Virus Res* 63:45–52.

Mao J, Hedrick RP, Chinchar VG. 1997. Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish iridoviruses. *Virology* 229:212–220.

Marcogliese DJ, Rodrigue J, Ouellet M, Champoux L. 2000. Natural occurrence of *Diplostomum* sp. (Digenea: Diplostomatidae) in adult mudpuppies and bullfrog tadpoles from the St. Lawrence River, Québec. *Comp Parasitol* 67:26–31.

Martin TR, Conn DB. 1990. The pathogenicity, localization, and cyst structure of echinostomatid metacercariae (Trematoda) infecting the kidneys of the frogs *Rana clamitans* and *Rana pipiens*. *J Parasitol* 76:414–419.

Matthews KR, Pope KL. 1999. A telemetric study of the movement patterns and habitat use of *Rana muscosa*, the mountain yellow-legged frog, in a high-elevation basin in Kings Canyon National Park, California. *J Herpetol* 33:615–624.

Matthews KR, Pope KL, Preisler HK, Knapp RA. 2001. Effects of non-native trout on Pacific treefrogs *(Hyla regilla)* in the Sierra Nevada. *Copeia* 2001:1130–1137.

Mazzoni R. 2000. Diseases in farmed American bullfrog (*Rana catesbeiana* Shaw, 1802) in Uruguay. In: Getting the jump! On amphibian diseases: proceedings; 2000 Aug 26–27; Cairnes, Queensland, AU. p 37.

McCallum H, Dobson A. 1995. Detecting disease and parasite threats to endangered species and ecosystems. *TREE* 10:190–194.

Meshaka WE. Exotic species and the conservation of North American Amphibians. In: Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Meteyer CU, Loeffler IK, Fallon JF, Converse KA, Green E, Helgen JC, Kersten S, Levey R, Eaton-Poole L, Burkhart JG. 2000. Hind limb malformations in free-living northern leopard frogs (*Rana pipiens*) from Maine, Minnesota, and Vermont suggest multiple etiologies. *Teratology* 62:151–171.

Meyer-Rochow VB, Asashima M. 1988. Naturally occurring morphological abnormalities in wild populations of the Japanese newt *Cynops pyrrhogaster* (Salamandridae; Urodela; Amphibia). *Zool Anz* 221:70–80.

Middleton EM, Herman JR, Celarier EA,Wilkinson JW, Carey C, Rusin RJ. 2001. Evaluating ultraviolet radiation exposures determined from satellite data at sites of amphibian declines in Central and South America. *Conserv Biol* 15:914–929.

Miera V, Davidson EW, Collins JP. 2002. Cutaneous chytridiomycosis in *Pseudacris triseriata.* Abstract *Sonoran Herpetological Society*.

Milius S. 1998. Fatal skin fungus found in U.S. frogs. *Science News* 154:7.

Moody NJG, Owens L. 1994. Experimental demonstration of pathogenicity of a frog virus, Bohle iridovirus, for a fish species, barramundi *Lates calcarifer*. *Dis Aquat Org* 18:95–102.

Morehouse EA, James TY, Ganley ARD, Vigalys R, Berger L, Murphy PJ, Longcore JE. 2003. Multilocus sequence typing suggests the chytrid pathogen of amphibians is a recently emerged clone. *Mol Ecol* 12:395–403.

Moyle PB. 1973. Effects of introduced bullfrogs, *Rana catesbeiana*, on the native frogs of the San Joaquin Valley, California. *Copeia* 1973:18–22.

Muths E, Corn PS, Pessier AP, Green DE. 2003. Evidence for disease-related amphibian decline in Colorado. *Biol Conserv* 110:357–365.

Mutschmann F, Berger L, Zwart P, Gaedicke C. 2000. Chytridiomykose bei Ambhibien—erstmaliger Nachweis für Europa. *Berliner und Münchener Tierärztliche Wochenschrift* 113:380–383.

Needham PR, Vestal EH. 1938. Notes on growth of golden trout (*Salmo aguabonita*) in two high Sierra lakes. *Calif Fish and Game* 24:273–279.

New Mexico Department of Game and Fish. 2001. Chiricahua leopard frog surveys; chytrid fungus brings deadly consequences. *Share with Wildlife* Spring–Summer 2001:6.

Nichols DK, Lamirande EW, Pessier AP, Longcore JE. 2001. Experimental transmission of cutaneous chytridiomycosis in dendrobatid frogs. *J Wildl Dis* 37: 1–11.

Nichols DK, Pessier AP, Longcore JE. 1998. Cutaneous chytridiomycosis in amphibians: an emerging disease? *Proc Am Assoc Zoo Vet* 269–271.

Nichols DK, Smith AJ, Gardiner CH. 1996. Dermatitis of anurans caused by fungal-like protists. *Proc Am Assoc Zoo Vet* 220–221.

[NWHC] National Wildlife Health Center. 2003. USGS National Wildlife Health Center Quarterly Mortality Reports. Available from: http://www.nwhc.usgs.gov/pub_metadata/qrt_mortality_report.html. Accessed 2003 May.

Ouellet M. 2000. Amphibian deformities: current state of knowledge. In: Sparling DW, Linder G, Bishop CA, editors. Ecotoxicology of amphibians and reptiles. Pensacola, FL, USA: SETAC. p 617–661.

Ouellet M, Bonin J, Rodrigue J, DesGranges J-L, Lair S. 1997. Hindlimb deformities (ectromelia, ectrodactyly) in free-living anurans from agricultural habitats. *J Wildl Dis* 33:95–104.

Parker BR, Schindler DW, Donald DB, Anderson RS. 2001. The effects of stocking and removal of a non-native salmonid on the plankton of an alpine lake. *Ecosystems* 4:334–345.

Parker JM, Mikaelian I, Hahn N, Diggs HE. 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). *Comp Med* 52:265–268.

Pessier AP, Nichols DK, Longcore JE, Fuller MS. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). *J Vet Diag Invest* 11: 194–199.

Pfenning DW, Loeb MLG, Collins JP. 1991. Pathogens as a factor limiting the spread of cannibalism in tiger salamanders. *Oecologia* 88:161–166.

Pilliod DS, Fronzuto JA. *Ambystoma macrodactylum* Baird, 1849; long-toed salamander. In: Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Pilliod DS, Peterson CR. 2001. Local and landscape effects of introduced trout on amphibians in historically fishless watersheds. *Ecosystems* 4:322–333.

Piotrowski JS, Annis SL, Longcore JE. 2001. Physiology, zoospore activity, and extracellular enzyme production of *Batrachochytrium dendrobatidis*, a chytrid pathogenic to amphibians. *Inoculum* 52:54.

Powell MJ. 1993. Looking at mycology with a Janus face: a glimpse at chytridiomycetes active in the environment. *Mycologia* 85:1–20.

Punzo F, Lindstrom L. 2001. The toxicity of eggs of the giant toad, *Bufo marinus*, to aquatic predators in a Florida retention pond. *J Herpetol* 35:693–697.

Raverty S, Reynolds T. 2001. Cutaneous chytridiomycosis in dwarf aquatic frogs (*Hymenochirus boettgeri*) originating from Southeast Asia and western toad (*Bufo boreas*) from northeastern British Columbia. *Can Vet J* 2001:385.

Read JL, Tyler MJ. 1994. Natural levels of abnormalities in the trilling frog (*Neobatrachus centralis*) at the Olympic Dam mine. *Bull Environ Contam Toxicol* 53:25–31.

Real L. 1996. Disease ecology. *Ecology* 77:989.

Real L, McElhany P. 1996. Spatial pattern and process in plant-pathogen interactions. *Ecology* 77: 1011–1015.

Reddacliff LA, Whittington RJ. 1996. Pathology of epizootic haematopoietic necrosis virus (EHNV) infection in rainbow trout (*Oncorhynchus mykiss* Walbaum) and redfin perch (*Perca fluviatilis* L). *J Comp Pathol* 115:103–115.

Reed KD, Ruth GR, Meyer JA, Shukla SK. 2000. *Chlamydia pneumoniae* infection in a breeding colony of African clawed frogs (*Xenopus tropicalis*). *Emerg Infect Dis* 6:196–199.

Relyea RA, Mills N. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (*Hyla versicolor*). *Proc Nat Acad Sci USA* 98:2491–2496.

Riggin Jr GT. 1956. A note on *Ribeiroia ondatrae* (Price, 1931) in Puerto Rico. *Proc Helminthol Soc Wash* 23:28–29.

Ron SR, Merino A. 2000. Amphibian declines in Ecuador: Overview and first report of chytridiomycosis from South America. *Froglog* 42-4. Available from: http://www2.open.ac.uk/biology/froglog/FROGLOG-42-4.html. Accessed 2003 May.

Rosen PC, Schwalbe CR.1995. Bullfrogs (*Rana catesbeianna*): introduced predators in southwestern wetlands. In: LaRoe ET, Farris GS, Puckett CE, Doran PD, Mac MJ, editors. Our living resources: a report to the nation on the distribution, abundance, and health of U.S. plants, animals, and ecosystems. Washington, DC, USA: U.S. Department of the Interior National Biological Service. p 452–454.

Rosen PC, Schwalbe CR, Parizek Jr DA, Holm PA, Lowe CH. 1995. Introduced aquatic vertebrates in the Chiricahua region: effects on declining native ranid frogs. In: DeBano LF, Folliott PF, Ortega-Rubio A, Gottfried GJ, Hamr RH, Edminster CB, tech. coords. Biodiversity and management of the madrean archipelago: the sky islands of southwestern United States and northwestern Mexico. Fort Collins, CO, USA: U.S. Department of Agriculture, Forest Service, Gen Tech. Rep. RM-GTR-264. p 251–261.

Rosen PC, Schwalbe CR, Sartorius SS, Bodenhamer HG, Cobble K, Magoffin M. Decline of the Chiricahua leopard frog in southern Arizona: Effects of non-native fishes and bullfrogs. Forthcoming.

Rostand J. 1949. Sur diverses anomalies relevées dans une population de crapauds (*Bufo bufo*). *C R Soc Biol* 143:758–760.

Rostand J. 1955. Nouvelles observations sur l'anomalie P chez la grenouille verte (*Rana esculenta* L.). *C R Soc Biol* 149:2075–2077.

Rostand J. 1958. Les anomalies des amphibiens anoures. Paris, FR: Société d'Édition d'Enseignement Supérieur (SEDES). 100 p.

Rostand J. 1971. Les étangs à monstres: histoire d'une recherche (1947-1970). Paris, FR: Stock. 85 p.

Rostand J, Darré P. 1968. Conditions déterminantes de l'anomalie P chez *Rana esculenta*. *C R Soc Biol* 162:1682–1683.

Sandlund OT, Schei PJ, Viken A. 1999. Introduction: the many aspects of the invasive alien species problem. In: Sandlund OT, Schei PJ, Viken A, editors. Invasive species and biodiversity management. Dordrecht, NL: Kluwer Academic Publishers. p 1–7.

Schell SC. 1985. Handbook of trematodes of North America north of Mexico. Moscow, ID: University Press of Idaho. 263 p.

Semlitsch RD. 2000. Principles for management of aquatic-breeding amphibians. *J Wildl Manag* 64:615–631.

Sessions SK, Franssen RA, Horner VL. 1999. Morphological clues from multilegged frogs: are retinoids to blame? *Science* 284:800–802.

Sessions SK, Ruth SB. 1990. Explanation for naturally occurring supernumerary limbs in amphibians. *J Exp Zool* 254:38–47.

Sexton OJ, Phillips C. 1986. A qualitative study of fish-amphibian interactions in 3 Missouri ponds. *Trans Missouri Acad Sci* 20:25–35.

Shaffer HB, Trenham PC. *Ambystoma californiense* Gray, 1853; California tiger salamander. In: Lannoo MJ, editor. Status and conservation of U.S. amphibians. Berkeley, CA, USA: University of California Press. Forthcoming.

Shields JD. 1987. Pathology and mortality of the lung fluke *Haematoloechus longiplexus* (Trematoda) in *Rana catesbeiana*. *J Parasitol* 73:1005–1013.

Snyder NFR, Derrickson SR, Beissinger SR, Wiley JW, Smith TB, Toone WD, Miller B. 1996. Limitations of captive breeding in endangered species recovery. *Conserv Biol* 10:338–348.

Sparling DW, Fellers GW, McConnell LL. 2001. Pesticides and amphibian population declines in California. *Environ Toxicol Chem* 20:1591–1595.

Sparrow FKS. 1960. Aquatic phycomycetes. 2nd ed. Ann Arbor: University of Michigan Press.

Speare R, Berger L. 2000a. Chytridiomycosis in amphibians in Australia. Available from: http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyspec.htm. Accessed 18 August 2003.

Speare R, Berger L. 2000b. Global distribution of chytridiomycosis in amphibians. Available from: http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm. Accessed 18 August 2003.

Speare R, Smith JR. 1992. An iridovirus-like agent isolated from the ornate burrowing frog *Limnodynastes ornatus* in northern Australia *Dis Aquat Org* 14:51–57.

Sredl M, Caldwell D. 2000. Update on chytrid surveys of frogs from Arizona. *Sonoran Herpetologist* 13:1.

Stebbins RC, Cohen NW. 1995. A natural history of amphibians. Princeton, NJ, USA: Princeton University Press.

Storfer AS, Collins JP, Mech SG, Reudink MW, Ziemba RE, Warren J. Evidence for introgression in the endangered tiger salamander, *Ambystoma tigrinum stebbinsi*. Forthcoming.

Taylor SK. 2001. Mycoses. In: Wright KM, Whitaker BR, editors. Amphibian medicine and captive husbandry. Malabar, FL, USA: Kreiger. p 181–191.

Taylor SK, Green DE, Wright KM, Whitaker BR. 2001. Bacterial diseases. In: Wright KM, Whitaker BR, editors. Amphibian medicine and captive husbandry. Malabar, FL, USA: Kreiger. p 159–179.

Taylor SK, Williams ES, Mills KW. 1999. Mortality of captive Canadian toads from *Basidiobolus ranarum* mycotic dermatitis. *J Wildl Dis* 35:64–69.

Taylor SK, Williams ES, Thorne ET, Mills KW, Withers DI, Pier AC. 1999. Causes of mortality of the Wyoming toad. *J Wildl Dis* 35:49–57.

Tweedell K, Granoff A. 1968. Viruses and renal carcinoma of *Rana pipiens* V. Effect of Frog Virus 3 on developing frog embryos and larvae. *J Natl Cancer Inst* 40:407–410.

Tyler T, Liss WJ, Ganio LM, Larson GL, Hoffman R, Deimling E, Lomnicky G. 1998. Interaction between introduced trout and larval salamanders (*Ambystoma macrodactylum*) in high-elevation lakes. *Conserv Biol* 12:94–105.

Tyler T, Liss WJ, Hoffman RL, Ganio LM. 1998. Experimental analysis of trout effects on survival, growth, and habitat use of two species of ambystomatid salamanders. *J Herpetol* 32: 345–349.

[USFWS] U.S. Fish and Wildlife Service. 2003. Endangered and threatened wildlife and plants: 12-month finding for a petition to list the Sierra Nevada distinct population segment of the mountain yellow-legged frog (*Rana muscosa*) as endangered. *Fed Reg* 68:2283–2203.

[USGS] U.S. Geological Survey. 2000 Aug. USGS Diagnoses Causes of Many U.S. Amphibian Die-Offs. Available from: http://www.usgs.gov/public/press/public_affairs/press_releases/pr1272m.html. Accessed May 2003.

[USNPS] U.S. National Park Service. 2001. Preliminary restoration of mountain yellow-legged frogs environmental assessment. Three Rivers, CA, USA: Department of Interior, National Park Service, Sequoia and Kings Canyon National Parks. 38 p.

Veith M, Viertel B. 1993. Veränderungen an den Extremitäten von Larven und Jungtieren der Erdkröte (*Bufo bufo*): Analyse möglicher Ursachen. *Salamandra* 29:184–199.

Waldman B, van de Wolfshaar KE, Klena JD, Andjic V, Bishop PJ, de B Norman RJ. 2001. Chytridiomycosis in New Zealand frogs. *Surveillance* 28:9–11.

Wassersug RJ. 1976. A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. *Stain Technol* 51:131–134.

Woïtkewitch AA. 1961. Le développement des extrémités surnuméraires chez les amphibiens. *Bull Biol Fr Belg* 95:569–600.

Wolf K, Bullock GL, Dunbar CE, Quimby MC. 1968. Tadpole edema virus: A viscerotropic pathogen for anuran amphibians. *J Infect Dis* 118:253–262

Wolf K, Bullock GL, Dunbar CE, Quimby MC. 1969. Tadpole edema virus: a viscerotropic pathogen for anuran amphibians. In: Mizell M, editor. Biology of amphibian tumors. New York, NY, USA: Springer-Verlag. p 327–336.

Woodwell GM. 1989. On causes of biotic impoverishment. *Ecology* 70:14–15.

Worthylake KM, Hovingh P. 1989. Mass mortality of salamanders *(Ambystoma tigrinum)* by bacteria *(Acinetobacter)* in an oligotrophic seepage mountain lake. *Great Basin Nat* 49:364–372.

World Conservation Union. 1998. Guidelines for re-introductions (as approved by the 41st meeting of the ICUN Council, 1995). Gland, CH: Re-introduction Specialist Group, World Conservation Union. 10 p.

Zardus M, Blank T, Schulz D. 1977. Status of fishes in 137 lakes in Sequoia and Kings Canyon National Parks, California. Three Rivers, CA, USA: U.S. Department of Interior, National Park Service, Sequoia and Kings Canyon National Parks.

Zhang QY, Li ZQ, Gui JF. 1999. Studies on morphogenesis and cellular interactions of *Rana grylio* virus in an infected fish cell line. *Aquaculture* 175:185–197.

Zhang QY, Li ZQ, Jiang YL, Kiang SC, Gui JF. 1996. Preliminary studies on virus isolation and cell infection from disease frog *Rana grylio*. *Acta Hydrobiol Sin* 20:390–392. (Chinese with English abstract).

Zhang QY, Xiao F, Li ZQ, Gui JF. 2001. Characterization of an iridovirus from the cultured pig frog *(Rana grylio)* with lethal syndrome. *Dis Aquat Org* 48(1):27–36.

Zupanovic Z, Musso C, Lopez G, Louriero CL, Hyatt AD, Hengstbverger S, Robinson AJ. 1998. Isoation and characterization of iridoviruses from the giant toad *Bufo marinus* in Venezuela. *Dis Aquat Org* 33:1–9

Zweifel, RG. 1955. Ecology, distribution, and systematics of frogs of the *Rana boylei* group. *Univ Calif Publ Zool* 54:207–292.