Epizootiology of Sixty-Four Amphibian Morbidity and Mortality Events in the USA, 1996–2001

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ABSTRACT: A total of 44 amphibian mortality events and 20 morbidity events were reviewed retrospectively. The most common cause of amphibian mortality events was infection by ranaviruses (Family: Iridoviridae). Ranavirus epizootics have abrupt onset and affect late-stage larvae and recent metamorphs. Mortality events due to ranavirus infections affected only widespread and abundant amphibian species, and there was a clear association with high population densities. Chytrid fungal infections accounted for seven mortality events in postmetamorphic anurans only. Chytrid epizootics are insidious and easily overlooked in the field. While both ranavirus and chytrid fungal epizootics were associated with >90% mortality rates at affected sites, only the chytrid fungal infections were linked to multiple amphibian population declines. Three primitive fungal organisms in the newly erected clade, Mesomycetozoa, caused morbidities and mortalities in anurans and salamanders.

KEYWORDS: amphibians; ranavirus; "red leg" disease; iridovirus infections; frogs; toads

INTRODUCTION

Amphibian population declines, extirpations of populations, and species extinctions have increased in the last 25 years.[1–5] While some declines, extirpations, and extinctions are easily attributed to loss of habitat, many have occurred in relatively protected, remote, or pristine locations. Some population declines, extirpations, and extinctions have been preceded by documented mortality events,[6–8] but the cause of most mortality events was not confirmed by appropriate diagnostic tests. Other amphibian species have declined and disappeared insidiously; it is unclear in these cases whether field casualties were missed because of insufficient monitoring, because of efficient removal of carcasses by scavengers, because sick amphibians died in cryptic refugia, or whether there were no morbidities or mortalities preceding the declines.

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Spontaneous morbidity and mortality events (MME) in free-living amphibians are infrequently and poorly documented. The majority of published mortality events in amphibians have been attributed to "red leg" disease associated with *Aeromonas hydrophila*, but nearly all reports prior to 1990 have no supporting histological findings, and virus cultures consistently were not attempted. In the United States, Wolf *et al.*[9] documented an iridovirus mortality event in bullfrogs (*Rana catesbeiana*) and demonstrated the susceptibility of numerous other amphibian species to the virus, which was named tadpole edema virus. Iridovirus epizootics were not reported in the United States for another 28 years until mortality events in Sonora tiger salamanders (*Ambystoma stebbinsi*) were documented in Arizona.[10] The seminal work of Cunningham *et al*.[11] correlated bacterial and virus cultures with histology and emphasized the importance and morphological variations of iridovirus infections in numerous mortality events in Great Britain.

Throughout the 1990s, amphibian mortality events in numerous countries were associated with worrisome and persistent population declines. Berger *et al*.[12] correlated mortality events in Panama and Australia with a newly recognized chytrid fungal infection of the amphibian epidermis. The fungus was identified and named *Batrachochytrium dendrobatidis*.[13] Epizootic chytridiomycosis has been detected in recent years throughout the United States and in some cases involved multiple declining and threatened species.[14–17]

In 1996, in response to public concerns about high malformation rates in young postmetamorphic amphibians in Minnesota, USA,[18] the National Wildlife Health Center, U.S. Geological Survey, expanded its diagnostic capability to include amphibians. With each succeeding year, increasing numbers of amphibian MME have been reported and investigated. Sixty-four amphibian MME investigated between 1996 and early 2001 are included in this retrospective epizootiological investigation. The causes of these MME are presented as well as epizootiological features of the major amphibian diseases.

MATERIALS AND METHODS

Pathology reports and associated epizootiological files on 64 amphibian MME were reviewed (TABLES 1 and 2). Only cases in which amphibians were submitted for diagnostic examination were selected. Each event or case consisted of one to 84 amphibians; some cases included a few sympatric fish, reptiles, and invertebrates. Cases that consisted of only one or two submitted amphibians were included in this study only if the species was in decline or if population survey data were available to indicate either prevalence of the disease or magnitude of population decline. Casualties involving amphibian eggs and embryos are excluded.

Routine diagnostic examinations of each amphibian casualty event included necropsy under a dissecting microscope, virus cultures on fathead minnow cell line at 25°C, aerobic bacterial cultures, parasite identification, and histological examinations. Submitters were interviewed (telephone or e-mail) by a wildlife disease specialist to determine onset of casualties, casualty numbers, population size, duration of event, sympatric species, and effect on population size.

TABLE 1. Amphibian morbidity events in the United States, 1996–2001, investigated by the U.S. Geological Survey, National Wildlife Health Center

Number	Affected spp.	Life Stage	Location	Onset Date	Estimated Sick	Disease or Diagnoses
1	Tiger salamander	Larva	North Dakota	August 1996	>95%	Dermal metacercariae[b]
2	Green frog	RM	Wisconsin	June 1997	2%	Malformations
3	Mink frog	RM	Minnesota	July 1997	Unk	Malformations
4	Northern leopard frog	Larva	Maine	July 1998	>75%	Intestinal coccidiosis
5	Northern leopard frog	RM	Minnesota	July 1998	Unk	Malformations
6	Grotto salamander	Larva	Missouri	August 1999	100%	Dermal metacercariae
7	Western toad, Pacific treefrog	Larva	Oregon	Est. July 1999	5%	Hypopigmentation, Giantism
8	Barton Spring salamander[a]	Larva	Texas	April 2000	10%	Malformations (scoliosis)
9	Spring salamander	Larva	Tennessee	March 2000	<5%	Dermosporidiosis
10	Santa Cruz long-toed salamander, California red-legged frog	Larva	California	May 2000	Unk	Trauma, Chytrid fungus

TABLE 1. Amphibian morbidity events in the United States, 1996–2001, investigated by the U.S. Geological Survey, National Wildlife Health Center —(*Continued*)

Number	Affected spp.	Life Stage	Location	Onset Date	Estimated Sick	Disease or Diagnoses
11	Western toad, Bullfrog, Pacific tree-frog, Foothills yellow-legged frog	Larva	California	June 2000	Unk	Dermal metacercariae, malformations & Chytrid fungus
12	Pacific giant salamander, Rough-skin newt	Larva & Adult	California	July 2000	<10%	Dermal metacercariae
13	Bullfrog	Larva	New Hampshire	July 2000	50%	Malformations, Iridovirus
14	Blue-spotted salamander	RM	Indiana	Nov. 2000	5%	Dermal metacercariae
15	American toad	Adult	Virginia	Nov. 2000	Unk	Suspect Dermosporidiosis
16	Pacific treefrog	RM	California	Dec. 2000	Unk	Malformations
17	Barton Spring salamander[a]	Larva	Texas	March 2001	10%	Dermal metacercariae
18	Red-spotted newt, Four-toed salamander	Adult	Tennessee	April 2001	<5%	Dermal filariasis
19	American toad	Adult	Virginia	May 2001	<5%	Dermosporidiosis
20	Green frog	RM	Vermont	August 2000	Unk	Ichthyophonus

ABBREVIATIONS: RM, recent metamorph; Unk, unknown (unreported).
[a]Barton Spring salamander, *Eurycea sosorum* n. sp.
[b]Dermal metacercaris were ususally identified as *Clinostomum* sp.

TABLE 2. Amphibian mortality events in United States, 1996–2001, investigated by the U.S. Geological Survey, National Wildlife Health Center

Number	Affected spp.	Life Stage	Location	OnsetDate	Estimated Dead	Etiology
1	Tiger salamander	Larva	Colorado	Aug. 1996	>200	Iridovirus
2	Tiger salamander	Larva	North Dakota	May 1998	>200	Iridovirus
3	Mink frog, Northern leopard frog	RM	Minnesota	June 1998	>200	Iridovirus
4	Spotted salamander	Larva	Maine	July 1998	100	Iridovirus
5	Tiger salamander	Larva	Utah	Sept. 1998	>200	Iridovirus
6	Bullfrog	RM	Indiana	June 1998	100	ND
7	Wood frog	Larva	North Dakota	July 1998	>200	Iridovirus
8	Bullfrog	Larva	Ohio	April 1999	50	Anchorworm
9	Bullfrog	Adult	Illinois	May 1999	20	Ichthyophonus
10	Wood frog	Larva	Maine	July 1999	50	Iridovirus
11	Northern leopard frog	Adult	Colorado	May 1999	20	ND
12	Tiger salamander	Larva	Idaho	June 1999	150	Iridovirus & selenosis
13	Wood frog	Larva	Maine	July 1991	>200	Iridovirus
14	Spotted salamander, Pickerel frog	Larva	Tennessee	June 1999	100	Iridovirus
15	Pickerel frog	RM	New Hampshire	July 1999	25	Iridovirus
16	Boreal toad	Adult	Colorado	Aug. 1999	20	Chytrid fungus

TABLE 2. Amphibian mortality events in United States, 1996–2001, investigated by the U.S. Geological Survey, National Wildlife Health Center —(*Continued*)

Number	Affected spp.	Life Stage	Location	OnsetDate	Estimated Dead	Etiology
17	Boreal toad	Adult	Colorado	May 1999	32	Chytrid fungus
18	Bullfrog	RM	Massachusetts	Aug. 1999	>200	Iridovirus
19	Bullfrog	Larva	New Hampshire	Sept. 1999	>200	Dermocystidium-like fungus
20	Tiger salamander	Larva	Wyoming	July 1999	>200	Iridovirus
21	Northern leopard frog	Adult	North Dakota	Nov. 1999	150	Chytrid fungus
22	Wood frog, Bullfrog, Spotted salamander	Larva	North Carolina	April 2000	>200	Iridovirus & Dermocystidium-like fungus
23	Southern leopard frog	Adult	North Carolina	Jan. 2000	Unk	Chytrid fungus
24	Tiger salamander	Larva	North Dakota	June 2000	>200	Iridovirus
25	Mink frog, Green frog, Northern leopard frog	Larva	Minnesota	June 2000	>200	Iridovirus & Dermocystidium-like fungus
26	Wood frog, Spotted salamander	Larva	Massachusetts	June 2000	>200	Iridovirus
27	Pickerel frog	RM	Maine	July 2000	100	Iridovirus
28	Mink frog	Larva	Minnesota	June 2000	>200	Iridovirus
29	Bullfrog	Larva	California	June 2000	>200	ND
30	Spring peeper	Larva	Maine	June 2000	>200	Iridovirus
31	Boreal toad	Adult	Wyoming	July 2000	Unk	Chytrid fungus
32	Green frog	Larva	Maine	Aug. 2000	>200	Iridovirus

TABLE 2. Amphibian mortality events in United States, 1996–2001, investigated by the U.S. Geological Survey, National Wildlife Health Center —(*Continued*)

Number	Affected spp.	Life Stage	Location	OnsetDate	Estimated Dead	Etiology
33	Mudpuppy	Adult	New York	July 2000	>200	ND
34	Bullfrog	Larva	Maine (Smith Pond)	Aug. 2000	>200	Iridovirus
35	Bullfrog	Larva	Maine (Cedar Lake)	Aug. 2000	>200	Iridovirus
36	Bullfrog	Larva	Maine (Peasant Lake)	Aug. 2000	>200	Iridovirus
37	Tiger salamander	Larva	Idaho	Aug. 2000	100	Iridovirus & selenosis
38	Mountain yellow-legged frog	RM	California	Aug. 2000	>200	Chytrid fungus
39	American toad, Bullfrog	Adult & larva	Maine	July 2000	100	ND
40	Wyoming toad	Adult	Wyoming	Sept. 2000	25	Chytrid fungus
41	California red-legged frog	Adult	California	Oct. 2000	Unk	ND, suspect chytrid
42	Wood frog, Red-spotted newt	Adult	Tennessee	Feb. 2001	100	ND, suspect weather
43	Southern leopard frog	Larva	Mississippi	March 2001	>200	Dermocystidium-like fungus
44	Wood frog	Adult	Maryland	May 2001	Unk	Ichthyophonus

ABBREVIATIONS: RM, recent metamorph; Unk, unknown (unreported); ND, not determined.

RESULTS

There were 20 morbidity events (disease outbreaks) involving 8 species of frogs, 2 species of toads, and 10 species of salamanders in 13 states (TABLE 1). In 15 cases (75%), only one species was involved, and in all cases, multiple species of amphibians were present at the site. The larval stage was the affected life stage in 55% of cases; postmetamorphs constituted the other half of cases. Recent metamorphs (those amphibians that completed metamorphosis within 30 days of capture) constituted 30% of morbidity events and were about twice as likely as adults to be involved in disease outbreaks.

Onset of nonlethal disease outbreaks in amphibians occurred from March to December; 55% of all cases were detected and submitted in a three-month time frame: June, July, and August; 30% of cases were detected and submitted in July. No disease outbreaks were recorded in January, February, September, and October, but three events occurred in November and December.

Fourteen amphibian morbidity events were attributed to only one etiology or disease process; multiple etiological agents were detected in six cases. The most common reason for submission of live amphibians was musculoskeletal deformities (malformations); it was the only cause for submission of six cases and was a factor in a mixture of etiologies in four other cases. The cause of musculoskeletal defects in most cases was not determined.

The second most common disease associated with morbidity events was infection by dermal metacercaria. The contributing biologist's principal concern or complaint in free-living amphibians was the presence of multiple distinct nodules and lumps in the skin. Dermal metacercaria, most often identified as *Clinostomum* sp., occurred in salamanders in five of six cases, and one case consisting of multiple species of tadpoles.

Small skin nodules, crusty ulcers, or skin pustules due to infection by *Dermosporidium* spp. were the main complaint in three cases, each consisting of only one affected amphibian from extensively surveyed populations. Hence, in each case, prevalence of infection was <5%. Two of three cases were first detected and submitted in the spring (March to May). Dermosporidiosis was not detected as an incidental, clinically silent, or lethal infection in the other amphibians (61 cases) in this study. Many amphibians with dermal metacercarial infections were submitted because dermosporidial infection was suspected.

Other diseases associated with amphibian morbidity events included runting in tadpoles due to intestinal coccidiosis, tail and limb trauma associated with invasive species of crayfish, swellings of the body wall associated with filarial nematodes, and swollen dorsal pelvic musculature due to infection by *Ichthyophonus fungus* (TABLE 1).

Mortality Events

The 44 mortality events are shown in TABLE 2. The number of recovered or dead amphibians counted in each case varied from 20 to several thousand. Seventeen species of amphibians were involved in these mortality events: 4 species of salamanders, 10 species of frogs, and 3 toad species. These mortality events occurred in 18 states. Seven die-offs involved two or more amphibian species, and multiple casualties in multiple species were suspected in another four die-offs (data not shown). Three distinct life stages of amphibians were involved in these die-offs: 25 (57%) events occurred principally in larvae; 6 (14%) involved mostly recent metamorphs (<30 days from completion of metamorphosis), and 12 (27%) events occurred mostly in adults. One mortality event involved a mix of larvae and adults. Most mortality events in larvae involved tadpoles and larval salamanders in late stages of development, such as Gosner stages 40 to 45,[19] which is a period of metamorphic climax. No mortality events involved recently hatched larvae (i.e., larvae <2 weeks posthatching).

The etiologies for these 44 amphibian mortality events were determined by virus, bacterial, and fungal cultures; toxicological analyses; and histological examinations. The main etiologies of these amphibian die-offs were (1) iridovirus infections (family, Iridoviridae; genus, *Ranavirus*), (2) combined iridovirus infection and selenium intoxication, (3) infection by the pathogenic amphibian chytrid fungus (*Batrachochytrium dendrobatidis*), (4) systemic infection by an unreported and previously unrecognized yeast-like fungal infection consistent with the mesomycetozoan organism, Dermocystidium, (5) fungal infections by a second mesomycetozoan, Ichthyophonus, (6) infection by the copepod, *Lernea* (anchorworms), and (7) not determined.

Iridovirus infections were the sole cause of 21 (48%) mortality events and were a factor in 4 (9%) additional die-offs with multiple etiologies. Recent metamorphs and larvae were the life stages involved in 24 of 25 mortality events in which iridoviruses were isolated or identified histologically. Iridovirus die-offs affected nine species of frogs and salamanders; only two amphibian species suffered iridoviral die-offs in the western half of the United States (west of 97th meridian), while eight species were affected in eastern states. In the western United States, each iridovirus mortality event involved only one amphibian species; seven western die-offs affected only larval tiger salamanders (*Ambystoma tigrinum*), and one case involved only wood frog tadpoles (*Rana sylvatica*). Multiple sympatric species of frogs and toads were present at western casualty sites involving tiger salamanders, but concurrent morbidities and mortalities in anurans were not observed. In 15 iridovirus-associated die-offs in Minnesota and four other eastern states, all events involved late stage tadpoles and recent metamorphs; three die-offs involved concurrent mortalities in frogs (tadpoles) and salamanders. Postmetamorphs of two months of age or greater were not affected in any iridoviral mortality events except one case in which multiple infectious agents were found.

Onset of each iridoviral die-off is shown in TABLE 2 and FIGURE 1. Twenty-two of 25 die-offs (88%) associated with iridovirus infections began in the months of June, July, and August. No iridoviral mortality events began in the months of October through March, and only one mortality event each occurred in April, May, and September. Daily casualty numbers in iridovirus die-offs are seldom available; however, casualty counts at each survey (data not shown) indicate the number of sick and dead amphibians ranged from as low as three to several hundred daily. Onset was often sudden; several biologists reported apparently normal populations on one day, and two or three days later, casualties consisted of hundreds or thousands of larvae. Duration of mortality events (data not shown) ranged from 5 to about 50 days; longer die-offs consistently involved multiple species of frogs, or frogs and salamanders. Total casualties at a site consistently exceeded 90%; recruitment was consistently zero or negligible.

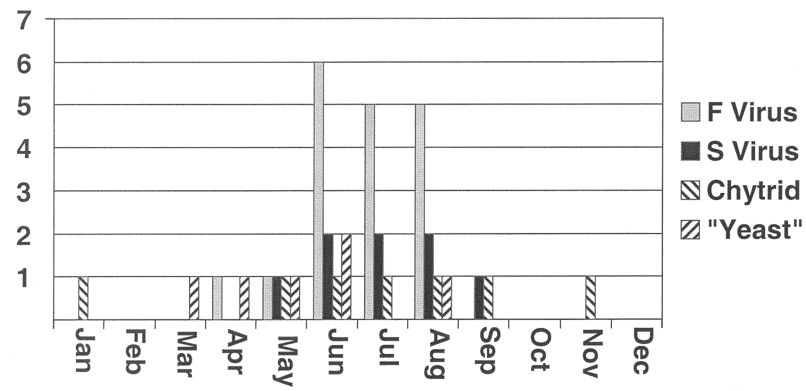

FIGURE 1. Date of onset of mortality events in amphibians due to four infectious diseases. F virus, ranavirus mortality events in frogs; S virus, ranavirus mortality events in salamanders; "Yeast," dermocystidium-like fungal mortality events. Y axis is the number of mortality events.

Iridovirus mortality events in multiple amphibian species have recurred for five consecutive years at one site in Minnesota, and massive die-offs of larvae for three consecutive years at sites in Tennessee and North Carolina are associated with iridovirus epizootics (not all years shown in TABLE 2, because submissions were solicited). There are insufficient annual field data on other mortality sites to estimate the probability of recurring annual die-offs at iridovirus casualty sites.

Chytrid fungus infections were considered the cause of seven mortality events in five states. Mortality events occurred only in frogs and toads; affected species included northern leopard frogs (*Rana pipiens*), southern leopard frogs (*R. sphenocephala*), mountain yellow-legged frogs (*R. muscosa*), boreal toads (*Bufo boreas*), and Wyoming toads (*B. baxteri*); the latter three species are considered in serious decline. Chytrid fungus-associated mortality events occurred only in postmetamorphic frogs and toads (recent metamorphs to breeding age adults); no mortality events involving larvae and salamanders were detected, although larval morbidity occurred.[17]

A temporal pattern of onset of chytrid fungal epizootics was not evident: the seven die-offs began on six different months. Daily casualty numbers were very low, often reported as one sick or dead amphibian per two or three visits (or surveys) of a site. The maximum casualty number in one season of surveys and collections at a site in Colorado was 32, but the population suffered a severe decline in 12–18 months. Population declines were >95% at three additional sites in Colorado and California with chytrid fungus infections in boreal toads and mountain yellow-legged frogs, respectively. Duration of chytrid fungus-associated mortality events could not be determined because date of onset often was undetected and daily casualty numbers usually were less than one. However, casualties due to chytrid fungal infection were usually detected in the second year, except where populations were extirpated. Numbers of breeding age adults in the first year after a chytrid epizootic usually were reduced by >90%. Recurrence of chytrid epizootics at a site probably is an inaccurate concept; chytrid epizootics probably persist or continue steadily from year to year.

A previously unreported severe systemic fungal infection was considered the sole cause of two mortality events and contributed to tadpole mortalities in two other die-offs in which multiple factors were present. The infection is identified by lethargic tadpoles with abdominal distension, and prominently enlarged and pale liver, spleen, pronephroi, and mesonephroi. Massive numbers of spherical, 6–9 micron diameter nonbudding yeast-like organisms are present in blood vessels, sinuses, sinusoids, and lymphatics. The organisms resemble the mesomycetozoan fungus, Dermocystidium. The infection occurred only in ranid tadpoles in Minnesota, Mississippi, North Carolina, and New Hampshire.

Onset of mortality events associated with dermocystidium-like mesomycetozoan fungi had no pattern; the four mortality events occurred in four different months. Mortalities in recently metamorphosed frogs and adult frogs were not observed at the two casualty sites in which the fungus was the only detected etiology, but at one site, no recent metamorphs were found following the die-off, and at the other site, numbers of recent metamorphs were severely reduced. These observations suggest that this fungal infection causes a >95% mortality rate in this population of tadpoles, and greatly impairs recruitment that year. Recurrences have not been investigated.

Ichthyophonus fungal infections, also a mesomycetozoan fungus, were the cause of two amphibian mortality events. In both cases, adult bullfrogs and wood frogs were found lethargic, unresponsive to prodding, and thin. Massive numbers of ovoid to elongate fungal organisms were present within myocytes throughout the body. Both mortality events occurred in May, but actual numbers of casualties were not reported. Other recent metamorphs and adult amphibians at the sites with much milder infections were considered normal appearing. Hence, Ichthyophonus fungal infection may occur more often as a endemic disease in a population, with morbidity exceeding mortalities. An insufficient numbers of die-offs attributed to Ichthyophonus have been investigated to determine its affects on populations and the likelihood of annual recurrence.

The cause of seven mortality events was not determined. In four cases, failure to determine the cause was attributed to the decomposed condition of the carcasses. Although intoxications were suspected in these events, toxicological analyses were consistently unrewarding. An additional two mortality events in tiger salamanders in Idaho were attributed to combined iridovirus infection and toxic levels of selenium in the tissues (selenosis). Only two mortality events of undetermined cause involved two or more species of amphibians and other vertebrates and invertebrates; all other die-offs involved one amphibian species. Distribution of mortalities by life stage showed four die-offs in adult amphibians only, one in adult and larval anurans, and one in recent metamorphs; hence, 71% of mortality events of undetermined cause affected postmetamorphic amphibians. Onset of mortality events of unknown cause ranged from February to October; four die-offs (57%) began in the months of June and July. Seven species were involved, with bullfrogs being involved solely or partially in three die-offs. Impact on the amphibian populations and recurrences have not been determined due to sufficient data and insufficient elapsed time since the die-offs.

Declining, threatened, or endangered (DTE) amphibians of six species, Santa Cruz long-toed salamanders (*Ambystoma macrodactylum croceum*), Wyoming toad

(*Bufo baxteri*), boreal toad (*B. boreas*), California red-legged frog (*Rana aurora draytoni*), foothills yellow-legged frog (*R. boylii*), and mountain yellow-legged frogs (*R. muscosa*) were involved in eight morbidity or mortality events (TABLES 1 and 2). Detected diseases in DTE amphibians in these events were chytrid fungus infection (five events), parasitism by dermal metacercaria (one case), and suspected trauma by introduced species of crayfish (one case); in one case involving *Rana aurora draytoni*, the cause was not determined because of advanced decomposition of the carcasses. Iridoviruses and mesomycetozoan fungi were not detected in any DTE amphibians involved in MME.

DTE species endemic to the eastern United States have not been submitted for diagnostic examinations or health screening. However, sympatric amphibians from three historic breeding sites of four eastern DTE amphibians, Blanchard's cricket frog (*Acris crepitans blanchardi*), flatwoods salamander (*Ambystoma cingulatum*), and gopher frogs (*Rana c. capito* and *R. c. sevosa*) have had infections by chytrid fungi, anchorworms, and mesomycetozoan fungi, but iridoviruses were not detected.

DISCUSSION

Five infectious diseases, consisting of one virus, three fungi, and a copepod parasite, were the sole cause or concurrent factor in 37 of 44 mortality events. Iridoviral and chytrid fungal etiologies were the cause of 32 (73%) of amphibian mortality events, and our findings are consistent with recent published literature.[10,12,20,21] However, literature prior to 1995 suggests that red leg disease due to *Aeromonas hydrophila* or other gram-negative bacilli has been the cause of most amphibian die-offs in the United States. Red leg disease was not the cause of any of 64 amphibian MME in this study, nor was it considered a contributing factor in any die-offs. This suggests that most published amphibian die-offs attributed to red leg disease, in which virus cultures and histological examinations were not reported, are suspect. Diagnoses in these 64 cases indicate red leg disease and other forms of bacterial septicemia are an infrequent cause of amphibian MME and, furthermore, suggest red leg disease may be a frequently misused and overused diagnosis in amphibian medicine and herpetological epizootiology.

Viruses in the family, Iridoviridae, genus, *Ranavirus*, were the most frequent cause of amphibian mortality events, accounting wholly or partially for 25 of 44 (57%) die-offs. Unlike reports from Europe[11,22] in which casualties involved adult ranids, all iridoviral mortality events in the United States involved late stage anuran and caudate larvae, and recent metamorphs. Die-offs of tiger salamanders and bullfrogs due to iridovirus epizootics have been reported previously;[9,10,20] this report documents iridoviral casualties in eight additional species, including spotted salamanders (*Ambystoma maculatum*), eastern red-spotted newt (*Notophthalmus viridescens*), northern leopard frogs, green frogs (*Rana clamitans*), mink frogs (*R. septentrionalis*), pickerel frogs (*R. palustris*), wood frogs, and spring peepers (*Pseudacris crucifer*).

Our data show that iridovirus mortality events occurred only in widespread and abundant amphibian species. To date, iridoviruses have not been isolated from species in decline, but many DTE species have not been examined in sufficient numbers to make firm conclusions. While iridoviral casualty rates at a site are usually >90%, recruitment is negligible, and annual recurrences are known, none of the affected amphibian species are considered in decline, nor have they achieved threatened or endangered status. Because all iridoviral die-offs of American amphibians to date have involved fairly widespread and abundant species, iridoviral epizootics may be a disease of dense populations and a hazard of crowding. Iridoviral die-offs in western states (west of the 97th meridian) consistently involved only one species, while die-offs in eastern states involved numerous species of anurans and caudates at the same site. This distinct pattern suggests that at least two species or strains of *Ranavirus* are involved in amphibian iridoviral mortality events.

In most regions of the United States, amphibians migrate and congregate at breeding ponds in the months of February through May. Only three iridovirus mortality events began in the months of April and May, and no iridoviral die-offs involved adult amphibians in breeding condition. These observations indicate that many adult amphibians may be resistant to, or have acquired immunity to, iridoviruses. However, the source of virus in massive mortality events involving thousands of larvae remains unknown. Recurrence of iridoviral die-offs at multiple sites for two to five consecutive years suggests there are carrier animals, or the virus persists in water, soil, or sediment. Because some amphibians, such as bullfrogs and green frogs are summer breeders and deposit eggs at a time when the larvae of spring-breeding amphibians are in metamorphic climax, and because it is known that the Lucke tumor herpesvirus of northern leopard frogs is shed in massive quantities only during spawning,[23] iridovirus carrier animals or persistently infected amphibians need to be investigated. Wolf *et al*.[9] observed an inverse relationship between age of tadpoles and mortality rates due to tadpole edema virus, and concluded that some individuals survive infections and develop immunity.

Key epizootiological features of iridoviral die-offs are the following: onset of die-offs usually is in the months of June, July, and August when many larvae are metamorphosing; the number of casualties per survey of a site varies greatly from less than 10 to several thousand; onset of die-offs usually is sudden: larval populations at a pond may appear normal on one day, and show massive casualties two days later; duration of iridoviral die-offs ranges from five days to as much as six weeks; longer die-offs tend to involve multiple amphibian species; die-offs affecting only one species generally produce casualties for 5 to 14 days with >90% reduction of the larval population.

Four distinct fungal diseases are documented. Two fungi (Ichthyophonus and Dermocystidium), which for many decades have had uncertain taxonomic status, have been placed in the newly erected clade, Mesomycetozoa.[24] It is proposed that a third amphibian fungal organism, known as *Dermosporidium penneri*,[14,25] also belongs in the clade, Mesomycetozoa. Dermosporidiosis was documented in three morbidity events involving adult, breeding age, American toads (*Bufo americanus*), and one larval spring salamander (*Gyrinophilus porphyriticus*); no deaths were associated with these cases. However, extensive casualties occurred in association with a previously unreported systemic disease caused by an organism resembling Dermocystidium or rosette agent of fish;[26,27] these mortality events were limited to ranid tadpoles; casualties were not observed in recent metamorphs and adult amphibians. Ichthyophonus—the second fungal organism in the clade, Mesomycetozoa—was the cause of two mortality events of unknown extent and one morbidity event. The morbidity event in recent metamorphs was characterized by frogs with a marked swelling of muscles and soft tissues around the urostyle similar to those previously reported in adult newts;[28,29] however, the swelling at the rump and urostyle was absent in adult frogs in the two mortality events, which suggests larvae and adults have different disease courses when infected by Ichthyophonus. Subclinical and nonlethal infections by Ichthyophonus were occasionally detected in other larvae, recent metamorphs, and adults (data not shown) and were interpreted as very mild incidental infections. Hence, the three Mesomycetozoa-like fungi caused three distinctive disease syndromes: Dermocystidium-like infection caused high mortality rates in ranid tadpoles only; Dermosporidium infections had low prevalences, no mortalities, and infections were limited to adult, breeding age toads and one larval salamander; infections by Ichthyophonus caused low mortality rates in adult ranids and intermediate prevalence rates with distinctive gross swellings of the rump region in recently metamorphosed ranids.

Chytrid fungal infections by the amphibian pathogen, *Batrachochytrium dendrobatidis*, caused mortalities only in postmetamorphic frogs and toads. Although chytrid fungal infections were observed in ranid tadpoles and salamander larvae, no mortalities and no die-offs attributed to chytrid infection were seen in salamanders and larval anurans. Mortality events due to chytrid fungal epizootics are insidious, persistent, and associated with serious and dramatic population declines in three western species: boreal toads, Wyoming toads, and mountain yellow-legged frogs. Because no more than one sick or dead amphibian was found at each survey of a site, chytrid fungal epizootics are considered an insidious mortality event that could easily be overlooked by experienced herpetologists; detection of chytrid mortality events usually requires repeated surveys of a site to detect just two casualties. Hence, chytrid fungal epizootics, because of their insidious nature, are a leading candidate for many additional unexplained amphibian population declines in the United States.

Intoxications associated with agrichemicals and other contaminants were not confirmed in any of 64 MME, but tissues from only a small number of amphibians were submitted for toxicological analyses. The only confirmed intoxications in this study were two die-offs of tiger salamanders in Idaho due to combined iridovirus infection and selenium poisoning. Both mortality events occurred in tailing ponds of phosphate mines, and it is not clear whether chronic selenosis impaired the immune systems of these populations and predisposed larvae to viral infections, or whether the iridovirus die-offs would have occurred in the absence of elevated tissue concentrations of selenium. Intoxications may have contributed to seven mortality events in which an etiology was not detected. Infectious diseases such as chytridiomycosis are just as likely to have contributed to these die-offs, because the submitted carcasses frequently were too decomposed for meaningful cultures and histological examinations.

An additional six distinct but general categories were used in morbidity events, including dermal metacercarial infections; skin nodules, pustules, or swellings; limb malformations; trauma; runting; and giantism. Although the metacercaria of multiple genera of trematodes infect the skin of amphibians, few genera produce nodules of sufficient size to be detected by the unaided eye in live amphibians. The most common dermal metacercaria to produce visible skin nodules is *Clinostomum* sp. Precise causes were not determined for some morbidity events, such as limb malformations, hypopigmentation, and gigantism.

In summary, numerous infectious diseases are implicated in amphibian MME in the United States, but red leg disease was not found. While ranaviruses, chytrid fungi, and a newly recognized mesomycetozoan fungal infection were the cause of 77% of mortality events, it is not clear whether any of these pathogens are endemic diseases or newly introduced pathogens at each casualty site, because there are no data from health surveys of normal-appearing amphibian populations with which to make comparisons.

CONCLUSIONS

- Three distinct infectious diseases accounted for 77% of 44 mortality events: these pathogens are Ranavirus, chytrid fungus (*Batrachochytrium dendrobatidis*), and a previously unreported Dermocystidium-like mesomycetozoan fungus.
- Mortality events in American salamanders usually occur where populations are dense and usually are due to iridovirus infections.
- In the eastern United States, iridovirus die-offs involve both anurans and caudates. In the western United States, iridovirus die-offs involve only one species, usually the tiger salamander.
- Iridoviral mortality events affected predominantly larval amphibians, usually those in metamorphic climax. Accordingly, iridoviral mortality events are observed primarily during late spring and summer when a population of larval cohorts is in metamorphic climax.
- Iridoviral mortality events have a sudden onset, and casualties number in the scores to thousands of amphibians daily.
- Iridoviral epizootics have not been associated with DTE species, but few DTE species have been examined, and further studies are warranted.
- Chytrid epizootics are insidious with field casualties, usually numbering only one amphibian per two or three surveys of a site. Hence, it is likely that many chytrid epizootics are undetected.
- Amphibian mortalities due to chytrid fungal epizootics have been observed only in postmetamorphic anurans. Mortalities in larval amphibians and caudates have not been detected.
- Larval anurans and caudates may be infected by chytrid fungi, but such infections produce morbidities only and are limited to the oral discs of tadpoles and tips of digits of larval caudates.
- Chytrid epizootics in the western United States have features of an introduced, highly lethal infectious disease to which amphibian populations have no innate resistance.
- Chytrid fungal epizootics are the only infectious disease currently associated with population declines of multiple species.

- Mortality events associated with Dermocystidium-like organisms have occurred only in ranid larvae in states east of the 97th meridian.
- Insufficient MME associated with Dermosporidium, Ichthyophonus, and anchorworms have been observed to make any conclusions.
- Red leg disease, commonly attributed to infection by *Aeromonas hydrophila*, was not documented as a factor in any of 64 MME in amphibians, thus calling into question many published reports of die-offs due to this disease in the twentieth century.

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