

Chytridiomycosis Widespread in Anurans of Northeastern United States

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ABSTRACT An emerging disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis* has been associated with morbidity, mortality, and extinction of species. Typically, researchers have detected *B. dendrobatidis* only when examining amphibians for causes of mortalities; few data exist on infection rates where mortalities are lacking. During May–September 2000–2002 we obtained amphibian specimens killed by vehicles and others collected at remote off-road sites throughout Maine, USA, and from federal lands in 5 states in the Northeast. We detected infected specimens, mostly green frogs (*Rana clamitans*), at 5 of 7 national wildlife refuges, a federal waterfowl production area, and Acadia National Park. Seven of 9 species, including all Ranidae species, were infected throughout Maine; rates ranged from 14.6% in American toads (*Bufo americanus*) to 25.7% in northern leopard frogs (*Rana pipiens*). We did not detect any infections in 50 eastern gray tree frogs (*Hyla versicolor*) or 21 spring peepers (*Pseudacris crucifer*). Species that hibernate in terrestrial habitats seem to have lower rates of infection than species that hibernate in aquatic habitats. Infections peaked in spring and autumn and were associated with air temperatures optimal for *B. dendrobatidis* growth. The relatively high infection rates among species without documented die-offs suggest that either losses have occurred undetected, that the fungus is endemic and species have attained a level of resistance to infections becoming lethal, or that climatic conditions of the Northeast have a role in preventing infections from being lethal. Data on prevalence and distribution of this chytrid fungus in the Northeast may be useful in modeling its origins and predicting long-term ecosystem effects involving anurans. (JOURNAL OF WILDLIFE MANAGEMENT 71(2):435–444; 2007)

DOI: 10.2193/2006-345

KEY WORDS amphibians, *Batrachochytrium dendrobatidis*, chytridiomycosis, diagnosis, ecology, Maine, Northeast, prevalence.

During the 1990s a chytrid fungus caused deaths of anurans in Queensland, Australia (Berger et al. 1998) and in western Panama (Lips 1999). Concomitantly, captive blue poison dart frogs (*Dendrobates azureus*) and White's tree frogs (*Litoria caerulea*) began dying in the United States at the National Zoological Park (Washington, D.C.; Pessier et al. 1999). Death of these captive frogs also was associated with epidermal infections caused by a heretofore unknown chytrid fungus described by Longcore et al. (1999) as a new species, *Batrachochytrium dendrobatidis*. Chytrids are members of the phylum Chytridiomycota, an early diverging lineage of fungi. They reproduce asexually by forming unwallied, motile spores propelled by a single posteriorly oriented flagellum. They require water to disperse. Experiments fulfilled Koch's postulates, which are criteria formulated by Robert Koch in 1884 that must be fulfilled to establish a causal relationship between an infective agent and a disease. Criteria are 1) the infective organism must be detected in all diseased animals but not healthy ones, 2) the organism must be isolated and grown in culture, 3) the organism must cause disease when introduced into a healthy animal, and 4) the infective organism must be re-isolated from experimentally infected animals. Experiments by Nichols et al. (2001) clearly documented that *B. dendrobatidis* caused the epidermal infections and that the fungus was capable of causing death of susceptible but healthy anurans.

Since the original reports, *B. dendrobatidis* has been associated with amphibian die-offs in Arizona, California,

and Colorado in the United States (e.g., Bradley et al. 2002, Muths et al. 2003, Briggs et al. 2005), Mexico (Lips et al. 2004), Spain (Bosch et al. 2001), New Zealand (Waldman et al. 2001), and South America (e.g., Ecuador; Ron and Merino 2000). Reports of infections by *B. dendrobatidis* in wild amphibian populations usually have been associated with die-off events (Berger et al. 1998) or after die-off events (Retallick et al. 2004). Links between *B. dendrobatidis* and declines of amphibian populations have been reviewed by Daszak et al. (2003), and Weldon et al. (2004) proposed that *B. dendrobatidis* originated in Africa and was disseminated by international trade in the African clawed frog (*Xenopus laevis*). Although *B. dendrobatidis* has been in northeastern North America at least since 1961 (Ouellet et al. 2005), little information exists on infection rates in anuran populations that lack known or recorded die-offs. Our interest in this aspect of chytridiomycosis was piqued when the first northern leopard frog (*Rana pipiens*) that we (J. Longcore, J. E. Longcore) collected in Orono, Maine was heavily infected with *B. dendrobatidis*.

Our primary objective was to determine if *B. dendrobatidis* was infecting amphibians in the Northeast, especially Maine and on federal lands (e.g., national wildlife refuges, Acadia National Park) where no records of die-offs existed. Because growth of the fungus is confined between 4–25° C (Piotrowski et al. 2004) and its motile spores are transmitted in water, we wished to determine if infections peaked during cool, wet weather and waned as temperatures rose. Secondarily, we wanted to determine if infection differed among species, localities, sexes, and age classes of species. In

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addition, we used this study to determine if detection rates differed between the methods of examining fresh, unstained tissue versus fixed, stained, and sectioned tissue.

STUDY AREA

We collected specimens at national wildlife refuges and other federal lands in Maine (i.e., Carlton Pond, Acadia National Park) and cooperators collected specimens on federal refuges in Massachusetts, New Hampshire, Vermont, and New York, USA. These states are within the Humid Temperate Domain, Warm Continental Division, Laurentian Mixed Forest Province, and Northern Hardwoods Forest Section or Northern Hardwoods–Spruce Forest Section ecoregions of the United States (Bailey 1978). The following biophysical description of the New England Piedmont (McNab and Avers 1994) generally depicts the area. The glacially scoured region had stony soils of Pleistocene till and stratified drift mantle, kames, eskers, drumlins, and lacustrine plains. Elevations ranged 300–1,900 m at areas along the Appalachian Mountain range. Six major rivers (e.g., Penobscot, St. John) originated in the region; thousands of lakes, ponds, streams, and beaver (*Castor canadensis*)-created wetlands provided habitat for an array of aquatic avifauna (e.g., American black duck [*Anas rubripes*], common loon [*Gavia immer*], amphibians, and beaver). Hardwood and conifer forest in large and small parcels covered 80% of the area with maple (*Acer* spp.), birch (*Betula* spp.), beech (*Fagus* sp.), oak (*Quercus* spp.), pine (*Pinus* spp.), spruce (*Picea* spp.), and balsam fir (*Abies balsamea*) that provided habitats for mammals (e.g., white-tailed deer [*Odocoileus virginianus*], moose [*Alces alces*], black bear [*Ursus americanus*], and raccoon [*Procyon lotor*]). Mean annual precipitation was 910–1,780 mm, and mean annual temperature was 3–7° C.

METHODS

Field Collections

To prevent inter-wetland transfer of infected materials, we rinsed boots and equipment with 5% bleach before entering each wetland. We collected specimens statewide in Maine during April 2000 by our own efforts and those of volunteers of the Maine Amphibian Monitoring Program. We attempted to collect specimens from some of the same refuges in both years. We asked volunteers who were conducting amphibian-calling surveys throughout Maine to collect specimens killed on roads. Volunteers recorded date collected, collector name, township of collection, and names of species and preserved specimens in 70% ethanol in Zip-lock® bags (S.C. Johnson & Son, Inc., Racine, WI). In 2001, lack of rain, especially in April and May, resulted in few specimens killed on roads. Because of this circumstance and the fact that specimens collected in 2000 were not as widely distributed as desired, especially in remote northwestern Maine, we collected specimens at sites away from paved roads where we lacked samples. We collected live specimens with a long-handled net and euthanized them with the amphibian anesthetic, Finquel® (tricaine methanesulfonate,

[MS222]; Argent Chemical Laboratories, Inc., Redmond, WA) at the rate of 8 g per 3.78 L of water. All collecting and handling of amphibians followed guidelines for the Institutional Animal Care and Use Committees of the United States Geological Survey, Patuxent Wildlife Research Center, and the University of Maine (no. A2001-09-01).

Laboratory Procedures

We examined external characters, such as size of tympanic membrane, size of toes on the front feet, and extent of toe-webbing to determine species and sex of mangled specimens. The presence of egg sacs often verified sex and age when the testes and ovary were unidentifiable. We measured snout-to-vent length and tarsus length to the nearest millimeter with a vernier caliper to associate size to age. We considered all specimens of species obtained before the beginning of their breeding to be adults (i.e., having existed in the environment ≥ 1 season). Some species with a 2-year or 3–4-year maturation period may be considered sub-adults relative to breeding, but we did not attempt to discern this distinction for the often-mangled specimens. We considered small specimens collected in large numbers following a species' breeding period as recently metamorphosed frogs (i.e., young of the yr). If we were uncertain of age, we listed it as unknown.

Researchers can diagnose chytridiomycosis in amphibian epidermal cells by microscopically examining fresh tissue. Infections appear as clusters of small (approx. 5–10- μ m diam), walled spheres within epidermal cells; usually some spheres contain thin, internal walls (Fig. 1). We placed excised webbing from between toes on each hind foot on a slide with distilled water under a cover slip and examined by scanning the epidermal surface at 100–400 \times . We used 1,000 \times magnification to verify light infections. J. E. Longcore verified fresh specimens identified as positive by J. Longcore. We did not examine fresh samples of skin from the pelvic area (i.e., pelvic patch between hind legs), which is also a site of infection.

Preparation of tissues for histology followed procedures of Berger et al. (1999). We excised strips (approx. 4 \times 6 mm) of toe-webbing from each hind foot (often the same tissues that we examined fresh), a strip of skin of the pelvic area and, in 2000, a few samples of skin from the tibiae, and placed samples in standard cassettes that we immersed in 70% ethanol. We examined the small sample of salamanders by preparing cross-sections of hind feet, and skin from the ventral side of the tail. We processed samples routinely; we cut 3 or 4 5- μ m-thick, nonconsecutive sections from tissues of each specimen and stained them with hematoxylin and eosin. We examined each slide with a light microscope at 400 \times and scanned each section for the usually distinctive thalli within epidermal cells. We attempted to quantify the degree of *B. dendrobatidis* infection by noting relative density and distribution of thalli among tissues examined. We recorded locations of infections for each tissue section and rated each slide for severity of infection as light (few scattered thalli on one section of tissue), moderate (a few clusters of thalli on >1 section of tissue), or heavy (many

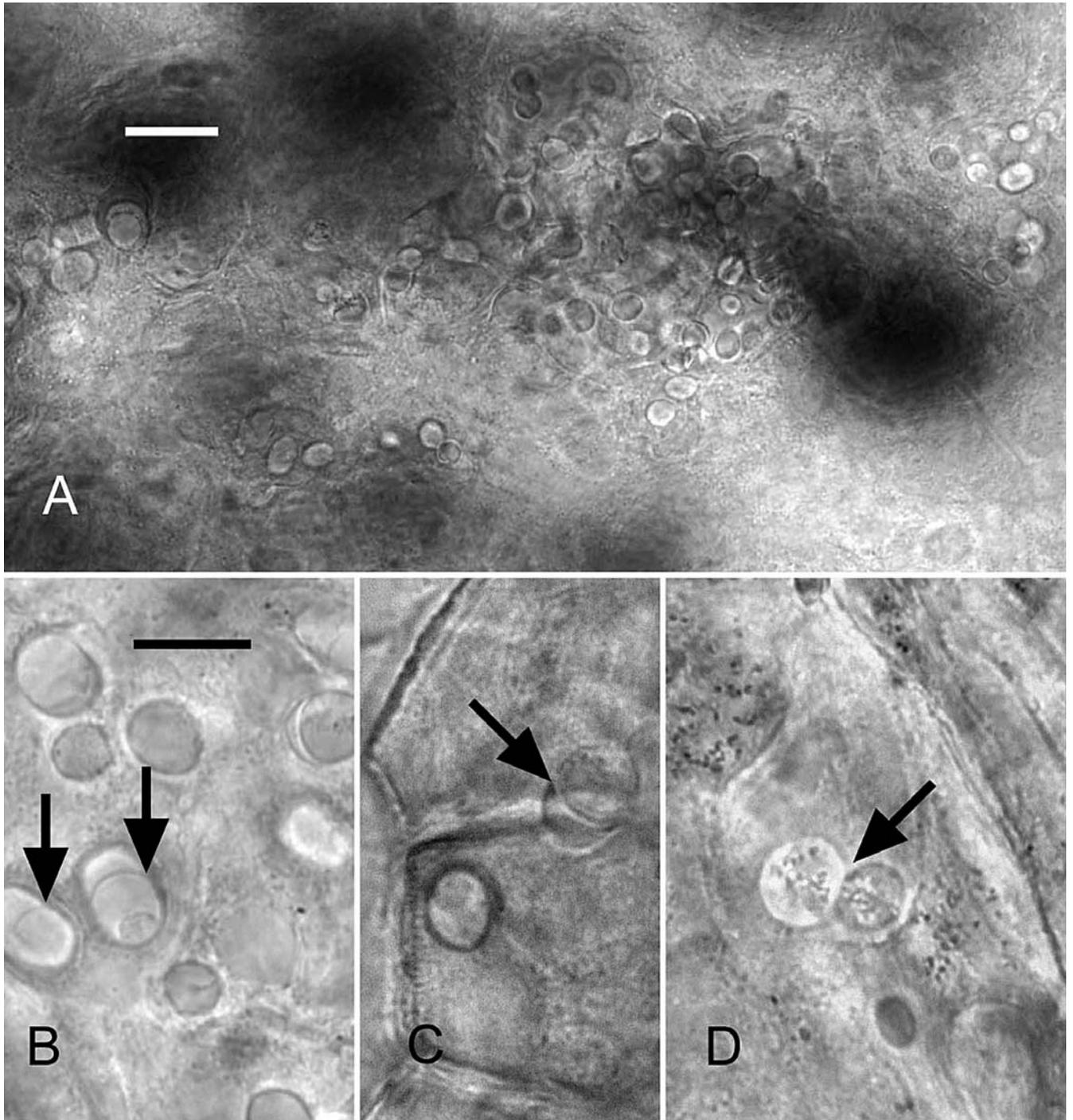


Figure 1. *Batrachochytrium dendrobatidis* thalli in fresh toe-webbing. A) View of infection in green frog tissue with 40× objective, the usual magnification for detection. Dark areas are melanin spots. B) Infection in green frog tissue with 100× objective. Arrows depict thalli with internal septa. C) Light infection in toe-webbing cells of bullfrog. Arrow points to internal septum. D) Light infection in crease of bullfrog toe-webbing. Thalli contain granules typical of those found in living cells (arrow). Measurement bars: A = 20 μm, B–D = 10 μm.

clusters of thalli, usually all sections of tissues infected). Our infection categories are relative among specimens we examined, and our heavy category does not correspond to the density of thalli in the stratum corneum of specimens that died from chytridiomycosis (Pessier et al. 1999). Pathologist A. Pessier verified infections identified as probable by J. Longcore. To evaluate reliability of fresh preparations versus histology of tissues, we conducted a

double-blind experiment to determine the error rate of histology. After examining fresh material, we stained and sectioned tissues from the same specimen for comparison. We (J. Longcore) examined those slides later and recorded infections without referring to the previous findings for the tissue when fresh.

We obtained weather records for the Bangor International Airport station (BGR), Bangor, Maine, (Lat. 44°49'N,

Table 1. Distributions of *B. dendrobatidis* infections among tissues of Maine, USA, anurans determined by histology, 2000–2002.

Species	Tissues examined								
	Toe-webbing			Tibiae skin			Pelvic skin		
	<i>n</i>	<i>n</i> infected	%	<i>n</i>	<i>n</i> infected	%	<i>n</i>	<i>n</i> infected	%
American toad	41	6	14.6	8	0	0.0	35	6	17.1
Bullfrog	141	15	10.6	31	1	3.2	128	8	6.2
Green frog	197	34	17.2	36	3	8.3	172	22	12.8
Pickerel frog	31	6	19.3	8	0	0.0	30	2	6.7
Northern leopard frog	71	14	19.7	21	0	0.0	68	7	10.3
Mink frog	102	17	16.7	2	1	50.0	101	9	8.9
Wood frog	45	5	11.1	25	2	8.0	59	4	6.8
Totals	628	97	15.5	131	7	5.3	593	58	9.8

Long, 68°49'W) from the National Oceanic Atmospheric Administration (NOAA) National Climatic Data Center to contrast air temperature differences among years, 2000–2002. We compared amounts of precipitation, frequency of rain events, and mean daily maximum temperature by month from April through September. We summed daily precipitation that exceeded a trace for each month, recorded the numbers of days per month with rainfall, and we used means from NOAA reports to present mean maximum temperatures per month each year (2000–2002).

Data Analyses

We designed our survey to determine presence or absence of *B. dendrobatidis* infections in species of amphibians killed on roads and collected by volunteers along random survey routes of the Maine Amphibian Monitoring Program. This approach was not suited to a formal sampling design because specimens of any given species were unpredictably scattered among many sites over time. Occurrence of killed amphibians on paved roads was affected by spotty rainfall and sporadic vehicular traffic. With additional, logistically constrained, off-road sampling, however, we collected enough useable specimens to test for differences in percentage of specimens infected among various response variables. We used a basic $2 \times k$ contingency table to identify overall differences among species, townships, and years with S-Plus statistical software (Insightful Corporation 2002). We then performed stepwise logistic regression to identify those entities (species, townships, yr) that contributed to differences; we used Pearson chi-square tests to determine significance. For those species with adequate numbers, we used chi-square tests to determine differences in percentage infected for variables sex, age (ad vs. recently metamorphosed frogs), and size (snout-to-vent length, a surrogate for age). Because *B. dendrobatidis* grows best at 23° C (Piotrowski et al. 2004) and temperatures can exceed that in summer, we evaluated by logistic regression the association between percent infected and date (i.e., Julian date) we collected the specimens. We used McNemar's test (Zar 1984) to evaluate differences in rate of detection between microscopically examining fresh tissue and stained sections of tissue for all species, all *Rana* species excluding the green frog (*Rana clamitans*), and for the green frog alone. We set an alpha level of 0.05 and because of only one degree of

freedom, we applied Yates correction for continuity, expressing the chi-square as χ^2_c .

We compared infection rates of terrestrial habitat hibernators (American toad [*Bufo americanus*], gray tree frog [*Hyla versicolor*], spring peeper [*Pseudacris crucifer*], and wood frog [*R. sylvatica*]) versus aquatic habitat hibernators (bullfrog [*R. catesbeiana*], green frog, pickerel frog [*R. palustris*], northern leopard frog, and mink frog [*R. septentrionalis*]) as suggested by P. S. Corn (United States Geological Survey, personal communication).

RESULTS

Critique of Detection Methods

Although the bodies of vehicle-killed amphibians examined during this study were often severely mangled, hind feet suffered less damage, which allowed us to collect data from numerous animals without killing healthy frogs. Among tissues examined by histology (i.e., webbing of hind toes, skin from tibiae, and skin from the pelvic patch), hind-toe-webbings consistently had higher infection rates (Table 1) than other tissues. When we examined toe-webbing from both hind feet by histology for *Rana* species, 70.8% (46 of 65) of the specimens were infected on webbing of both feet. Of the 205 *Rana* specimens that had webbing from both feet examined by histology, 19 of 205 (9.3%) were infected on one foot and 46 of 205 (22.4%) were infected on both feet.

Because we found so few (5.3%) specimens with infections in skin from the tibiae, we did not sample this tissue after the first year. Infections in the pelvic patch were about half as prevalent as those in toe-webbings for most species, except for the American toad in which 6 specimens positive for toe-webbings were also infected in the pelvic patch. Heavy infections seem typical of chytridiomycosis in bufonids.

Relative to scanning fresh tissue with a light microscope versus scanning stained and sectioned tissue, the largest discrepancy was for green frogs, with 11.1% more infections detected by scanning fresh tissue than by scanning stained sections. Rates of detection by the 2 methods were the same for pickerel frogs and mink frogs. Neither method detected infections all the time; 21 samples that we scored positive when we examined fresh tissue were falsely determined

Table 2. Infections of *B. dendrobatidis* in hind-toe-webbings of Maine, USA, anurans as determined by histology and by scanning of fresh tissues of the same specimens at 400–1,000× with a light microscope, 2000–2002.

Species	<i>n</i>	Histology		Fresh	
		No.	% infected	No.	% infected
American toad	11	0	0	0	0
Gray tree frog	11	0	0	0	0
Spring peeper	7	0	0	0	0
Bullfrog	68	12	17.6	15	22.0
Green frog	81	23	28.4	32	39.5
Pickerel frog	23	6	26.1	6	26.1
Northern leopard frog	50	13	26.0	14	28.0
Mink frog	19	6	31.6	6	31.6
Wood frog	29	5	17.2	6	20.7
Totals	299	65	21.7	79	26.4 ^a

^a The proportion of tissues detected as infected was greater ($P < 0.05$; McNemar's test) by examining fresh tissue than by scanning stained sections, but most of the difference was attributed to the green frog.

negative by histology. Seven samples that we scored negative when we missed sparse thalli while examining fresh tissue, we determined positive by histology.

For a sample ($n = 299$) of toe-webbings that we examined by both methods, observation of fresh tissue yielded 4.7% more infections than did examination of histology slides ($\chi^2_c = 6.03$, 1 df, $P < 0.05$; Table 2). This difference was related to the greater rate of infection for the green frog alone ($\chi^2_c = 4.09$, 1 df, $P < 0.05$) because the infection rate of all other species combined was not different ($\chi^2_c = 1.07$, 1 df, $P > 0.05$) between methods. Among all species, the percent infected was consistently higher for toe-webbing (10.6–19.7%) than for skin from the tibia (0.0–8.3%, except 1 of 2 mink frogs examined was infected) or tissue from the pelvic patch (6.2–17.1%; Table 1).

The percent of infected individuals that we scored as a light infection ranged from 28.6–76.9% among species, whereas we scored 16.7–42.8% as a moderate infection and 2.6–50.0% as a heavy infection (Table 3). We designated 50% of the infections as heavy in American toads, whereas we scored only 2.6% of the infections as heavy in bullfrogs, and we scored 76.9% as light.

Table 3. Severity of *B. dendrobatidis* infections in hind-toe-webbings of Maine, USA, anurans as determined by histology and by scanning of fresh tissue with a light microscope, 2000–2002.

Species	<i>n</i>	Severity of infections ^a					
		Light		Moderate		Heavy	
		No.	%	No.	%	No.	%
American toad	6	2	33.3	1	16.7	3	50.0
Bullfrog	39	30	76.9	8	20.5	1	2.6
Green frog	52	24	46.1	10	19.2	18	34.6
Pickerel frog	18	8	44.4	4	22.2	6	33.3
Northern leopard frog	19	9	47.4	5	26.3	5	26.3
Mink frog	8	3	37.5	3	37.5	2	25.0
Wood frog	7	2	28.6	3	42.8	2	28.6
Totals	149	78	52.3	34	22.8	37	24.8

^a Light infection: few scattered thalli on one section of tissue; moderate: few clusters of thalli on >1 section of tissue; heavy: many clusters of thalli, usually on all sections of tissues.

Regional and Maine Infection Rates

Sixty-one (13.6%) of 447 specimens were infected at 5 national wildlife refuges and on other federal lands in the Northeast; we did not find any infected specimens at Iroquois National Wildlife Refuge (NWR) in New York or Missisquoi NWR in Vermont (Table 4). Of the total sample 22.9% (36 of 157) of adults, 8.9% (24 of 268) of recently metamorphosed frogs, and 4.5% (1 of 22) of age-unknown frogs were infected. Although we detected epidermal hyperplasia and hyperkeratosis, possibly consistent with a chytrid infection, in 2 green frogs at Iroquois NWR, the lack of infections in other specimens of that sample precluded a conclusive diagnosis of *B. dendrobatidis* infection. Green frogs were most often infected; we found positive specimens at 7 of 13 federal locations sampled (Table 4).

In the statewide sampling in Maine, we examined 11 vehicle-killed salamander specimens; none were infected: blue-spotted salamanders (*Ambystoma laterale*; $n = 1$), spotted salamanders (*A. maculatum*; $n = 7$), and eastern newts (*Notophthalmus viridescens*; $n = 3$). Throughout Maine, including on federal refuges, we collected 751 usable specimens of frogs and toads during 2000–2002. Percentages of species infected for combined years ranged from 0.0% for eastern gray tree frogs and spring peepers to 25.7% for northern leopard frogs (Table 5). The 100.0% without infections for eastern gray tree frogs ($\chi^2_1 = 22.5$, $P \leq 0.001$) and spring peepers ($\chi^2_1 = 10.0$, $P = 0.002$) was different from all other species examined, but all other species were not different from each other ($P \geq 0.105$). For all species combined, the percentage of specimens with evidence of infection was lower in 2001 than in either 2000 or 2002 ($\chi^2_1 = 14.5$, $P = 0.001$). In any given month, 0.8–2.3 cm more precipitation fell in 2000 than in 2001, except for July when 2.8 cm more rain fell in 2001, which was the drier year. Mean maximum temperatures increased from April through August and declined in September (Fig. 2).

In 2000, 257 of 315 (81.6%) specimens were adults with 22.2% infected, whereas in 2001, 133 of 348 (38.2%) were adults and 17.3% were infected. For combined years, the percent with infections that we detected declined ($\chi^2_1 = 6.2$,

Table 4. Infections by *B. dendrobatidis* in anurans at National Wildlife Refuges in the Northeast and at other federal lands in Maine, USA, 2001–2002.

State, refuge, or federal agency		Species ^a , no. examined - no. infected								
		AMT	SPP	GTF	BUF	GRF	PIF	NLF	MIF	WOF
MA	Great Meadows	6 - 0		1 - 0	1 - 0	23 - 2	1 - 0	14 - 0		5 - 0
ME	Aroostook	1 - 0						2 - 1	66 - 10	
	Moosehorn		2 - 0	5 - 0	25 - 3	13 - 6	2 - 1	1 - 1		1 - 1
	Petit Manan					1 - 1				
	Rachel Carson	1 - 0		1 - 0		38 - 2				
	Sunkhaze	2 - 0	4 - 0	9 - 0	13 - 2	23 - 11	2 - 1	32 - 3		5 - 2
	Carlton Pond ^b				6 - 0	6 - 3	10 - 0			
	Acadia Natl. Park			1 - 0	12 - 4		3 - 0			
NH	Great Bay					30 - 4				
	Umbagog					1 - 0	1 - 1			
NY	Iroquois ^c					14 - 0		19 - 0		
VT	Missisquoi							28 - 0		
	Silvio O. Contee (Nulhegan Division)				15 - 2				1 - 0	

^a AMT, American toad (includes one Fowler's toad [*Bufo fowleri*] for Great Meadows); GTF, gray tree frog; BUF, bullfrog; GRF, green frog; PIF, pickerel frog; NLF, northern leopard frog; MIF, mink frog; WOF, wood frog.

^b A Fish and Wildlife Service-designated Waterfowl Production Area.

^c No definite thalli detected, but pathology in 2 green frog specimens was possibly consistent with a chytrid infection but inconclusive (A. Pessier).

$P = 0.01$) with date of collection. We did not detect differences in percent infected between sexes ($\chi^2_1 = 4.97$, $P = 0.08$) or ages (ad vs. recently metamorphosed frogs; $\chi^2_1 = 3.62$, $P = 0.16$). Another approach to examining effects of age is to relate specimen size (i.e., snout-to-vent length) for each sex within each species. This approach also proved nonsignificant ($\chi^2_{39} = 47.7$, $P = 0.159$). The infection rate for the terrestrial hibernators was 8.3% (13 of 157) and for the aquatic hibernators it was 22.9% (136 of 594; Fig. 3).

Spatial patterns of infected specimens among Maine townships revealed that infected toads and frogs were widely distributed throughout the state. Fifty-one percent (37 of 73) of the townships sampled contained infected specimens (Fig. 4). In townships where we did not detect infected specimens, we collected only 1–3 specimens. When we collected 4–11 specimens per township, 53.6% of the townships contained infected specimens and all 12 of the townships with ≥ 12 specimens per township contained infected specimens (Fig. 5). Near an isolated beaver dam (approx. 4 km from paved road) in a township in northern Maine, 16 of 17 recently metamorphosed green frogs and one recently metamorphosed pickerel frog that we collected on a single date were infected.

DISCUSSION

We found that *Batrachochytrium dendrobatidis* is widespread in the Northeast. It occurred at all Maine sites where we examined at least 12 amphibians, at all national wildlife refuges in Maine, and at national wildlife refuges in New Hampshire, Massachusetts, and Vermont. This fungus infected all but 2 of the 9 species of anurans found in Maine, and those 2 species were primarily arboreal. This pattern of regional occurrence is expanded by data of Ouellet et al. (2005) who found, for the same 9 species that we examined, that the combined 1960–2001 rate of infection for Québec was 20.3%; this is comparable to our Northeast United States rate of 19.8% for 2000–2002. We conclude

Table 5. Chytridiomycosis in amphibians throughout Maine, USA, during 2000–2002, based on light microscopy and histology of toe-webbing, tibiae skin, and pelvic patch tissue.

Family and species	Yr	<i>n</i>	No. infected	% infected
Bufonidae				
American toad	2000	38	6	15.8
	2001	3	0	0
	Total	41	6	14.6
Hylidae				
Gray tree frog	2000	43	0	0
	2001	7	0	0
	Total	50	0	0
Spring peeper	2000	19	0	0
	2001	2	0	0
	Total	21	0	0
Ranidae				
Bullfrog	2000	41	7	17.1
	2001	73	10	13.7
	2002	65	22	33.8
	Total	179	39	21.8
Green frog	2000	75	27	36.0
	2001	111	21	18.9
	2002	18	4	22.2
	Total	204	52	25.5
Pickerel frog	2000	13	5	38.5
	2001	17	3	17.6
	2002	5	0	0
	Total	35	8	22.9
Northern leopard frog	2000	42	17	40.5
	2001	32	2	6.2
	Total	74	19	25.7
Mink frog	2000	2	1	50.0
	2001	100	17	17.0
	Total	102	18	17.6
Wood frog	2000	42	7	16.7
	2001	3	0	0
	Total	45	7	15.5

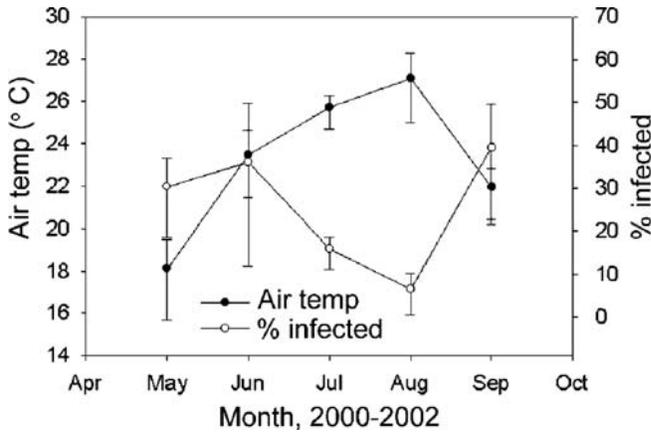


Figure 2. Mean (SE) maximum temperature ($^{\circ}$ C) of ambient air at Bangor, Maine, USA, for May–September 2000–2002 and mean (SE) percent of anurans infected by *B. dendrobatidis*.

that *B. dendrobatidis* likely infects amphibians throughout northeastern North America.

Several explanations are possible for the lack of observed declines in anuran populations despite significant rates of infection by a fungus that can be lethal (Berger et al. 1998, Nichols et al. 2001, Carey et al. 2006). *Batrachochytrium dendrobatidis* seems to be a clonal fungus with little genetic variation that is spreading around the world (Daszak et al. 1999, Morehouse et al. 2003). It is possible that existing anurans are more resistant to effects of the pathogen now than when it reached this area sometime before 1961, the collection year of the first *B. dendrobatidis*-positive specimen in the Northeast (Québec; Ouellet et al. 2005). The similar rates of infection in Australia (Retallick et al. 2004) in amphibians that have reestablished after a die-off event support this idea; however, the literature contains no reports of an initial wave of the disease in the Northeast. Perhaps, the particular species of anurans in northeastern North America are less susceptible to severe chytridiomycosis than

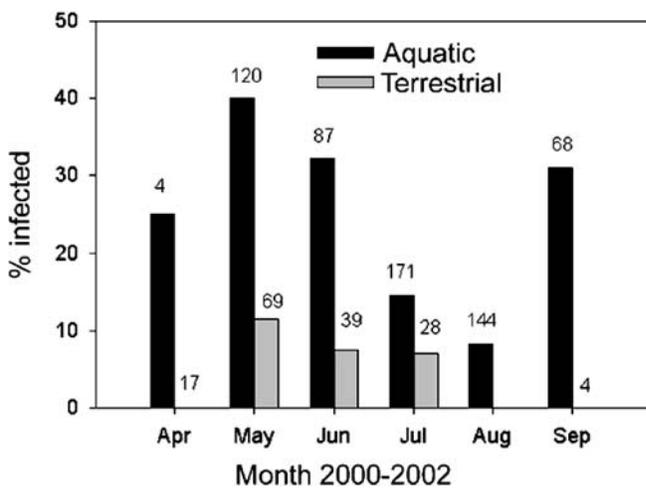


Figure 3. Monthly infection rates of anuran species that hibernate in aquatic and in terrestrial habitats in Maine, USA, 2000–2002. Samples sizes for each group by month are above bars.

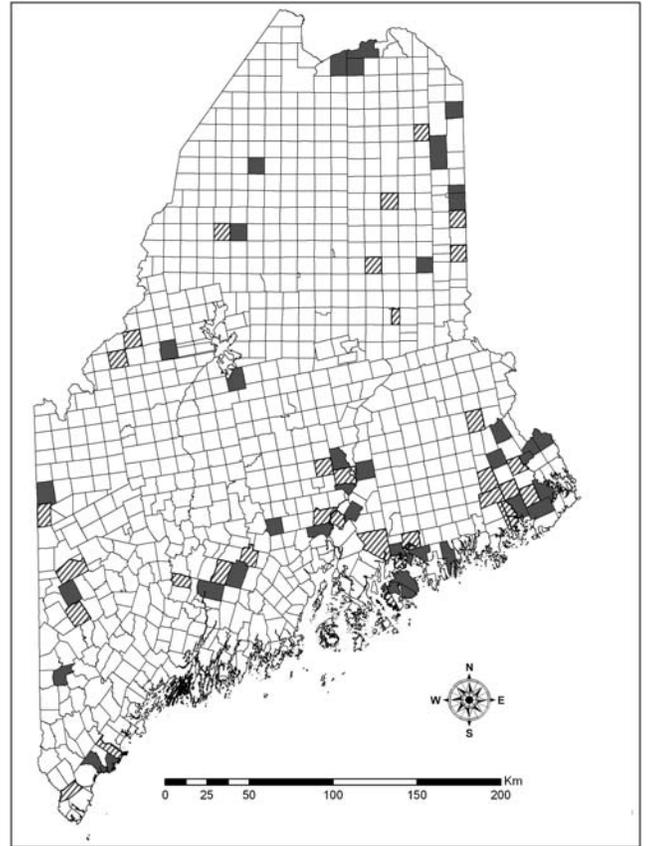


Figure 4. Distribution of *B. dendrobatidis* in amphibians in Maine, USA, townships, 2000–2002. White indicates no specimens collected in township; stripes indicate specimens collected in township but none infected; black indicates townships with infected specimens.

are those in areas where population declines have occurred. This is probably true for the bullfrog; even inoculation with millions of zoospores results in only minor infections (Daszak et al. 2004). Our histological and light microscopy data support the lack of heavy infections in this species. Two

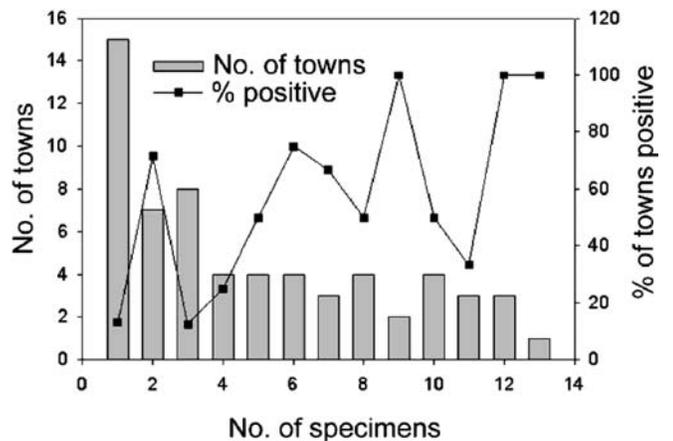


Figure 5. Number of specimens collected from individual Maine, USA, townships and percent of townships with *B. dendrobatidis*-infected specimens, 2000–2002. Data for townships with 1–13 specimens are plotted; all townships ($n = 15$) with ≥ 12 specimens were positive with ≥ 1 infected specimen.

other *Rana* species in the Northeast, green frog and mink frog, are closely related to the bullfrog and, based on mitochondrial DNA characters, are in the same subgenus (*Aquarana*; Hillis and Wilcox 2005). Although they have not been tested experimentally, it is possible that these 2 species also have innate resistance to severe infections by *B. dendrobatidis*. The mink frog, the most aquatic species in the region, however, had relatively heavy infections. Other species may be resistant because of their life histories. We did not detect *B. dendrobatidis* in gray tree frogs or spring peepers; however, both species are susceptible to infection. Ouellet et al. (2005) detected *B. dendrobatidis* in 1 of 16 gray tree frogs collected during 1964–2000 in Québec and an infected spring peeper was recorded in Louisiana at Atchafalaya National Wildlife Refuge in 2002 (L. Irwin, United States Fish and Wildlife Service, personal communication). The fact that tree frogs are arboreal and can take in dew and rainwater on vegetation (Stille 1958) where *B. dendrobatidis* zoospores are probably absent may lessen their exposure to infection. Similarly, low infection rates (15.5%) in wood frogs may relate to the fact that they spend much of their time out of water in upland sites (Regosin et al. 2003) where they are less exposed to zoospores. Lips et al. (2003), in discussing population declines in Central America, stated that the probability of decline increased with lifetime aquatic index (i.e., an average coded value for habitats designated as exclusively terrestrial, occupying multiple habitats, or exclusively riparian that are used by adults and larvae of a species) and body size. It seems that in the Northeast anuran species that spend less time in the water, including during hibernation, are less apt to be infected with *B. dendrobatidis*.

The highest prevalence of *B. dendrobatidis* in a Maine species was for northern leopard frogs, a species of special concern in Maine (Hunter et al. 1999) and of regional concern in the Northeast (Therres 1999). In Canada northern leopard frogs are ranked as endangered in British Columbia, of special concern in Alberta, Saskatchewan, and Manitoba but not considered at risk in eastern Canada (Alvo and Oldham 2000), where 10.7% of tested museum specimens collected between 1960 and 2001 in Québec were infected (Ouellet et al. 2005). Frogs in the southwestern species *R. chiricahuensis* and *R. yavapaiensis* in the leopard frog complex (*Pantherana*; Hillis and Wilcox 2005) have died with chytridiomycosis (Bradley et al. 2002). The northern leopard frog declined precipitously from unknown causes in Wisconsin the 1970s (Hine et al. 1981); deaths were associated with reddening of the ventral side of the legs, which was also seen in *R. yavapaiensis* from Arizona (Bradley et al. 2002) that died with heavy infections of *B. dendrobatidis*. These observations lead us to conclude that the northern leopard frog may be susceptible to lethal effects of chytridiomycosis under some circumstances.

Temperature influences the rate and severity of infection of anurans by *B. dendrobatidis* (Berger et al. 2004), and the pattern of temperatures experienced by anurans in the Northeast differs from that in the montane tropics where

some *B. dendrobatidis*-related amphibian declines have taken place (e.g., Berger et al. 1998, Lips et al. 2006). In the montane tropics temperatures for *B. dendrobatidis* are suitable year-round, whereas in northeastern North America ambient temperatures vary from low enough to high enough to limit growth of *B. dendrobatidis*, with periods suitable for growth limited to 2-month periods interrupted by temperatures high enough to limit growth (approx. 28° C; Piotrowski et al. 2004). Throughout the time of amphibian activity, thermoregulatory behavior may allow frogs to raise their body temperature to levels that will retard the growth of the fungus. Temperatures above those optimum for the chytrid can retard the time to death in species that are susceptible to lethal effects from chytridiomycosis (Berger et al. 2004). Basking on sunny days during cooler weather and summer temperatures in the Northeast may provide sufficient thermal setbacks to the pathogen that it does not cause noticeable population effects to species such as the northern leopard frog that might under some circumstances be susceptible to lethal effects from infection.

Although we did not detect differences in infection rates between males and females, between adults and recently metamorphosed frogs, or size class (a surrogate for age), Lamirande and Nichols (2002) found in experimental studies that newly metamorphosed anurans were more susceptible to the effects of chytridiomycosis than larger frogs. Consequently, the climatic conditions (both temp and cloud cover; Pounds et al. 2006) when infected larvae metamorphose may control whether amphibians die at this critical stage. Deaths of metamorphic anurans could be easy to miss and, thus, not be recorded.

Lack of historical population data for amphibians in the Northeast limits our ability to assess the effects of this or other diseases. In an exception to this, Gibbs et al. (2005) resurveyed amphibian habitats that were censused in New York from 1973–1980. They found that populations of 5 anuran species associated with roadside wetlands in western, central, and northern New York have remained stable in wetlands that are still extant. We did not detect *B. dendrobatidis* in either 14 green frogs or 19 northern leopard frogs collected at Iroquois National Wildlife Refuge in western New York, the only New York refuge for which we had collections. Data from spring amphibian calling surveys in Maine are recent (started in 1998) but will establish a baseline of population status so that changes can be assessed. Considering innate differences among species in their susceptibility to infection, the effects of temperature and the effects of life stage, we believe that understanding the effects of *B. dendrobatidis* on amphibians will require infection experiments for individual species at various temperatures and at various ages (e.g., Lamarinde and Nichols 2002).

MANAGEMENT IMPLICATIONS

We recommend that field personnel disinfect all equipment with a bleach solution when moving from site to site (Johnson et al. 2003) to prevent transfer of the fungus from

wetland to wetland. Because of the many anuran species infected with *B. dendrobatidis* on federal refuges in the Northeast, we recommend a formal monitoring program to detect anuran mortalities and infection rates. For routine monitoring of *B. dendrobatidis* infections, we recommend sampling fresh tissue from toe-webbing of hind feet of green frogs or northern leopard frogs in spring or autumn when infection rates are highest. When sampling American toads, we recommend that tissue from the pelvic patch be examined by histology. Although we did not collect anurans at fish hatcheries, anurans infected with *B. dendrobatidis* associated with fish at hatcheries could be a source of infection because hatchery water is widely dispersed throughout the state when fish are stocked. Although amphibian populations in the Northeast do not seem to be declining because of *B. dendrobatidis*, transfer of amphibians from Maine to other regions of the United States or countries should be preceded by testing for chytridiomycosis (Annis et al. 2004, Boyle et al. 2004). Furthermore, to evaluate the effects of infections by *B. dendrobatidis*, we need information on the status and trends of Maine amphibian populations. Data collected by the Maine Amphibian Monitoring Program since its inception should be analyzed soon. Our expanding knowledge of *B. dendrobatidis* as an emerging disease of amphibians is exposing a complex interplay of variables, especially of temperature, phylogeny of species, and life-history characteristics (e.g., over-wintering mode). Our data on *B. dendrobatidis* may be useful in developing predictive models that can forecast effects of this important amphibian pathogen. Changing climatic conditions may have profound effects on future infection rates and intensity of chytridiomycosis. Although populations of amphibians in the Northeast do not seem to be declining now, changes caused by global warming could alter this balance (e.g., Pounds et al. 2006).

ACKNOWLEDGMENTS

J. D. Taylor and L. E. Eaton-Poole provided liaison with National Wildlife Refuges and the primary funding. S. Hitchcock-Gallo and A. J. K. Calhoun provided contact with volunteers of the Maine Amphibian Monitoring Program (MAMP). E. Albert, L. Alverson, R. R. Bryan, P. G. deMaynadier, G. V. Flagg, S. Flagg, J. P. Gibbs, G. F. Higgins, T. P. Hodgman, C. L. Hudson, G. M. Inman, R. Kennedy, G. Kruse, M. McCollough, D. Phillips, L. A. Rowse, S. Smith, W. Weaver, and son D. Weaver collected specimens. T. Ayer, A. Densmore, M. C. Langois, K. M. Munney, L. E. Eaton-Poole, L. J. Welch, and especially S. E. Mierzykowski collected specimens in Maine and on National Wildlife Refuges in the Northeast. A. M. Gorman-Gelder provided tissue from mink frogs she was studying. D. A. Manski, B. A. Connery, and E. Flores provided specimens from Acadia National Park. K. J. Babbitt provided specimens from Maine and New Hampshire refuges. J. Kanter arranged for a collecting permit in New Hampshire, and G. R. Pozzuto approved one for White Mountain National Forest. R. W. McDiarmid

advised on metrics for age and sex determinations. D. J. Beane, University of Maine, provided most of the histology services; personnel at the University of Illinois provided some histology support. D. E. Green and G. S. McLaughlin provided diagnostic service. D. A. Clugston and P. O. Corr provided data summary. A. L. Meehan prepared Figure 4 and P. G. deMaynadier commented on a draft of the manuscript. National Science Foundation grants DEB no. 0213851 and DEB no. 9978094 to J. E. Longcore and DEB no. 0213851 to A. Pessier supported aspects of this research.

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Associate Editor: Chamberlain.