

***Batrachochytrium dendrobatidis*: Chytrid disease**

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Aquatic Invasion Ecology

Final Report

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## Section 1. Diagnostic information

*Batrachochytrium dendrobatidis*, commonly called chytrid disease, belongs to the Kingdom Fungi, Phylum Chytridiomycota, Class Chytridiomycetes, Order Chytridiales. It has not yet been assigned a family name.

*B. dendrobatidis* is a zoosporic fungus, belonging to a broader group known as the chytrid fungi. Chytrids lack hyphae (fungal filaments) and are ubiquitous in aquatic habitats and moist soil, where they break down cellulose, chitin, and keratin (Berger et al. 1999). Until recently parasitic chytrids were only known to infect plants, algae, protists, and invertebrates. *B. dendrobatidis* is the only chytrid known to infect vertebrates. It grows within the superficial keratinized layers of its host's epidermis, known as the stratus corneum and stratum granulosum (Daszak et al. 1999). The normal thickness of the stratus corneum is between 2 to 5  $\mu\text{m}$ , but infection with *B. dendrobatidis* can cause this layer to thicken to as much as 60  $\mu\text{m}$ . Chytrid can also infect the mouthparts of tadpoles, which are keratinized, but not skin of tadpoles, which lack keratin (Daszak et al. 1999). Although chytrid occurs only within keratinized tissues, it is unknown if *B. dendrobatidis* actually degrades keratin. It has been suggested it may be found in keratinized cells because these cells are dead and easier to invade (Piotrowski et al. 2004). The fungus produces roughly spherical, smooth-walled zoosporangium (zoospore- containing

bodies) 10 to 40  $\mu\text{m}$  in diameter (Berger et al. 2005). The zoospores contained within the zoosporangium are released from a discharge tube, which is plugged until the zoospores mature. Once mature the plug is shed and the zoospores are released. Chytrid zoospores are elongate to ovoid in shape and range from 0.7 to 6  $\mu\text{m}$  in diameter. A single posterior flagellum provides the zoospore with locomotion, allowing it to swim in water (Berger et al. 2005).

Techniques to identify *B. dendrobatidis* include histopathology (Briggs and Burgin 2004), immunohistochemical staining (Berger et al. 2002; Van Ells et al. 2003), histochemistry (Olsen et al. 2004), electronic microscopy (Berger et al. 2002), and real-time quantitative PCR TaqMan assay (Hyatt et al. 2007).

Histological preparation and microscopic examination of an amphibian's epidermis are common methods used to identify chytrid disease, but swab qPCR has gained acceptance internationally as a diagnostic tool to assess the presence of *B. dendrobatidis* (Smith 2007). qPCR can detect a single chytrid zoospore, and currently there is debate regarding the actual number of chytrid zoospores required to constitute an "infection" (Smith 2007). Testing of animals suspected to be infected with the disease usually involves toe clipping, water baths and filtration, and swabbing the skin of the individual, then extracting the DNA from that sample for use in the PCR reaction (Hyatt et al. 2007).

*Symptoms of chytridiomycosis (the disease caused by B. dendrobatidis infection)*

Amphibians infected with *B. dendrobatidis* typically display abnormal posture, lethargy, and lost righting reflex. Abnormal epidermal sloughing also occurs, occasionally accompanied by epidermal ulceration; hemorrhages in the skin, muscle, or eye; hyperemia of digital and ventrum skin, and congestion of viscera (Daszak et al. 1999).

Chytrid disease is fatal to many, but not all amphibians (Retallick et al. 2004). The mechanism by which chytrid causes death has not been elucidated but may include: 1) an impairment of cutaneous respiration or osmoregulation, 2) release of a fungal toxin that is absorbed systemically by the host, or 3) by a combination of these factors (Daszak et al. 1999)

## **Overview**

According to the Global Amphibian Assessment (2004) nearly one-third (32%) of the world's amphibian species are threatened, representing 1,896 species. The potential loss of this many amphibian species represents one of the greatest threats to vertebrate biodiversity in recorded history. While habitat loss is to blame for the decline of many species, amphibians continue to die out in undisturbed habitats. These more mysterious losses vex researchers, who ask: Why would populations in pristine areas also die out?

A massive detective effort now underway suggests a disease-causing fungus named *Batrachochytrium dendrobatidis*, called chytrid for short, lies at the center of this mystery. Found in Europe, Australia, the Americas and Africa, the disease (referred to as chytridiomycosis) is most devastating to amphibian populations in mountainous areas, where it thrives on moist cool conditions. Chytrid is implicated in the disappearance of many regional populations of frogs, and it has driven some endemic species to extinction. In one montane region of Australia it is believed 67% of the 110 species of harlequin frogs (*Atelopus spp.*) have died out in the past 20 years as a result of chytridiomycosis. Similarly, *B. dendrobatidis* caused a massive die-off in the Fortuna region of Panama, and it is probably responsible for the extinction of the golden toad (*Bufo periglenes*) there. Many of the chytrid-related die-offs are undocumented, due to remoteness of the regions where the disease flourishes.

Features of chytrid's biology (such as its low host selectivity) are enigmatic, and appear to favor its rapid spread, with and without human assistance. Worse yet, many invasive amphibian species such as the African Clawed Frog (*Xenopus spp.*), the American Bullfrog (*Rana catesbeiana*) and the Cane Toad (*Bufo marinus*) carry the disease, but do not succumb to it. Thus, as they spread, so does *B. dendrobatidis*. These species are part of a thriving international trade for use as pets and in human consumption.

Neither trade is regulated to stem the spread of chytrid disease. Furthermore, many of the issues related to the origin, distribution, mechanism of spread, and overall impact of chytrid fungus are highly debated. While these features are disputed, chytrid continues to move.

Chytrid fungus occurs in the Pacific Northwest (PNW), although its distribution has only been described Oregon. Its role in the declines of PNW amphibians is, as of now, unknown. In this paper I attempt to summarize the current research on the ecology, distribution, invasion dynamics, and impacts of *B. dendrobatidis*. Management efforts are underway to contain the spread of the disease but currently there no known way to control it once it moves into an area of susceptible amphibians.

## Origin and distribution

### History of invasiveness

The earliest known cases of *B. dendrobatidis* infection date back to 1938 in museum specimens of African clawed frogs

(*Xenopus spp.*) (Weldon et al. 2004). Twenty-three years elapsed before the first positive specimen was found outside of Africa. Weldon and colleagues (2004) believe the global shipment of African clawed frogs disseminated the disease worldwide. These frogs are asymptomatic when infected with the fungus.

Beginning in 1934 large numbers of *Xenopus spp.* were shipped throughout the world for use in pregnancy testing. Individuals of this species were injected with female urine, and if the frog produced eggs the test was considered positive. Even after this method of testing was abandoned, *Xenopus spp.* continued to be shipped worldwide as a model for scientific studies of immunology, and later embryology and molecular biology (Weldon et al. 2004). Feral populations of *Xenopus spp.* established in Ascension Island (South Atlantic Ocean), the United Kingdom, the United States, and Chile in 1944, 1962, the 1960s, and 1985, respectively. The first identified case of chytridiomycosis occurring outside of South Africa was isolated from *Rana clamitans* from Saint-Pierre-de-

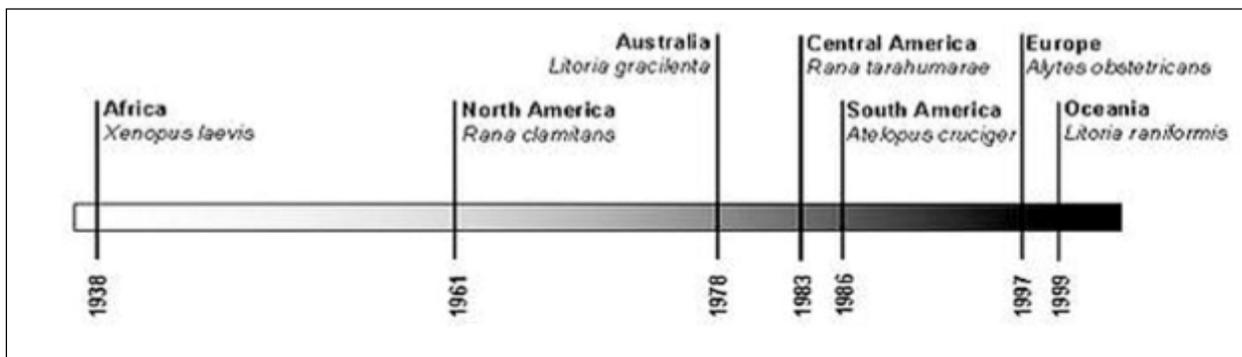


Figure 1. Time bar indicating the first occurrence of chytrid disease in each continent where it is present (Source: Weldon et al. 2004).

Wakefield, Québec, Canada, in 1961 (Figure 1, Weldon et al. 2004).

It is hypothesized that *Xenopus spp.* frogs moved the disease out of Africa, and from there, began to infect native amphibian species. Secondary spread of chytrid likely occurred with human-mediated movement of the American bullfrog (*Rana catesbeiana*). *R. catesbeiana* can acquire the disease, but does not show any clinical pathology as a result of the infection (Garner et al. 2006). This species was and still is shipped worldwide as part of a global food trade. The first chytrid infected *R. catesbeiana* dates back to 1978 in South Carolina (Weldon et al. 2004).

There is some debate as to whether *B. dendrobatidis* causes dramatic declines in amphibians because naïve anuran populations are being exposed to it for the first time (the “emerging disease” or “spreading pathogen” hypothesis) or whether some environmental shift has made amphibians more susceptible to the disease that was already present (the “endemic pathogen” hypothesis) (Daszak et al. 1999; Ouellet et al. 2005; Skerratt et al. 2007).

Molecular evidence suggests chytrid is a recently emerged clone spreading to naïve populations (Morehouse et al. 2003). This study supports the contention that *B. dendrobatidis* was recently introduced into two areas (one in Panama and one in Australia), where it caused massive amphibian mortality. Testing of museum specimens shows chytrid has been present in other areas such as North America

since at least the 1960s (Ouellet et al. 2005). It is likely that *B. dendrobatidis* caused declines in these populations long before anyone began looking at the fungus as a potential causative agent. The fungus was not even identified until 1998 (Daszak et al. 1999; Longcore et al. 1999). The competing hypothesis (using museum specimens as evidence) contends that chytrid is endemic to many regions, and that climate or other factors have altered the host-pathogen relationship, resulting in recent outbreaks of chytridiomycosis (Morehouse et al. 2003; Weldon et al. 2004).

### **Global native and non-native distribution**

*B. dendrobatidis* has been found on five continents including North and South America, Europe, Oceania (including Australia and New Zealand), and Africa. Thus far, it has not been found in Asia.

It is difficult to classify a “native” and “non-native” distribution for chytrid fungus, since it appears to be a recently emerged species, or strain. If Weldon et al. (2005) are correct, then *B. dendrobatidis* gained worldwide distribution from Africa with the shipment of the African clawed frog beginning in the 1930s (see above). I was unable to find a global map of chytrid distribution. Researchers Rick Speare and Lee Berger maintain a list of the global distribution of chytridiomycosis on the web ([http://www.jcu.edu.au/school/phtm/PHTM/frog\\_s/chyglob.htm](http://www.jcu.edu.au/school/phtm/PHTM/frog_s/chyglob.htm)), although it has not been updated

since 2004. The following account of the global distribution of *B. dendrobatidis* is taken largely from that website and from Ouellet et al. (2005). This is surely an underestimate of chytrid distribution, since extensive surveys for the fungus have not been conducted worldwide.

Australia has the greatest number of documented species infected with the fungus (46 species). There it can be found in three zones: 1) extending along the entire east coast of Australia from Cairnes to Melbourne, 2) a zone around Adelaide in South Australia, and 3) a south-west zone ranging from Perth to Albany. Chytrid is also found in New Zealand (Christchurch, South Island, and Coromandel Range, North Island).

In Africa *B. dendrobatidis* occurs in Kenya (Langata, Nairobi) and South Africa (Grabouw, Bredasor, Hex River, Knysna). In Central America chytrid occurs in Costa Rica (Rivas, San Isidro del General, and the central southern part of Costa Rica), Mexico, and Panama (Fortuna). *B. dendrobatidis* has been found in North America in the United States (the San Rafael Valley of Arizona, the Sierra Nevadas in California, the Southern Rocky Mountains of Colorado, Illinois, Indiana, Minnesota, North Carolina, South Carolina, Virginia, Wisconsin, Wyoming) and five Canadian provinces (British Columbia, Ontario, Quebec, New Brunswick, Nova Scotia) (Figure 2). In South America chytrid infections have been reported from Ecuador (the Riobamba and Azuay Provinces), Uruguay (Montevideo), and Venezuela (Estado Carabobo). The first outbreaks of

chytridiomycosis in Europe were reported from Spain, in Peñalara Natural Park, near Madrid in 1997-1999. It has also been reported in Italy (dinatomi di Bologna), and from captive animals in Germany (Berlin).

### **Current distribution in the Pacific Northwest**

Researchers have not yet conducted an exhaustive survey for *B. dendrobatidis* in the Pacific Northwest. The only published study to date describes the presence of *B. dendrobatidis* in 37 sites opportunistically sampled in Oregon (Pearl et al. 2007). Two sites in Olympic National Park in Washington were also sampled for chytrid in that study. Other researchers have conducted spot sampling for chytrid fungus, and have found it in Oregon and Washington (Blaustein personal communication).

*B. dendrobatidis* is widespread geographically and taxonomically in Oregon (Figure 3). The Northern Red-Legged Frog (*Rana aurora*), the Columbia Spotted Frog (*Rana luteiventris*), the Oregon Spotted Frog (*Rana pretiosa*), and the nonnative American Bullfrog (*R. catesbeiana*) all carry the disease. Of the 210 individuals screened for the disease in Pearl's 2007 study, 28% were positive. Positives were found across Oregon State, in the Upper Columbia River basin, the Upper Deschutes basin, and the Willamette and Umpqua basins. Chytrid was not found in the two sites sampled in Olympic National Park in Washington State.



Figure 2. Distribution of *B. dendrobatidis* in North America, 1895 to 2001. Source: Ouellet et al. 2005.

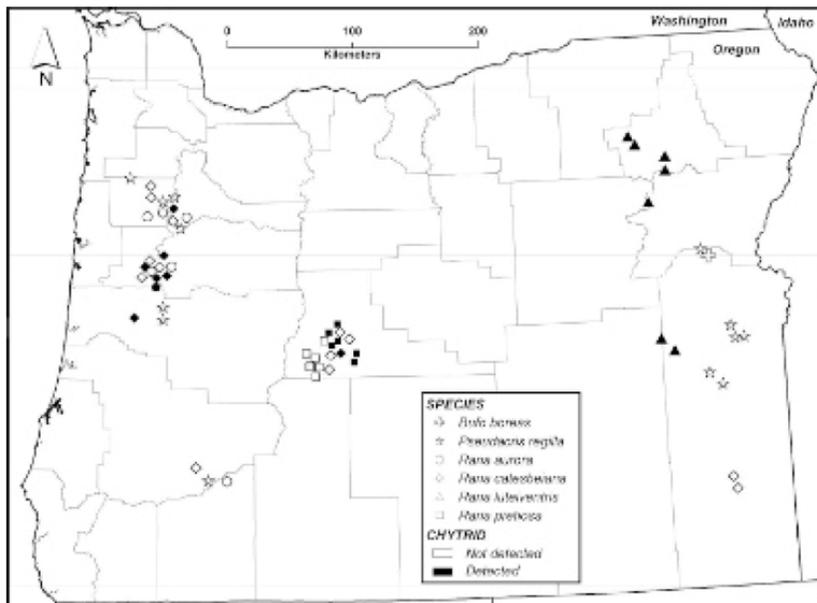


Figure 3. Detections of *B. dendrobatidis* in 37 sites sampled in Oregon. Filled symbols indicate at least one detection of the fungus. Source: Pearl et al. 2007.

## Life history and basic ecology

### Life cycle

*B. dendrobatidis* is an aquatic organism with two life history stages (Figure 4, Berger et al. 2005). The reproductive zoosporangium stage is sessile. Zoosporangium produce the second life history stage, called the zoospore, which once released from the zoosporangium, is motile in water via a single posterior flagellum. The zoospore directly attaches itself to the keratinized layers of its host. Once attached, it matures into a zoosporangium with rhizoids (filamentous extensions used for attachment and assimilation) (Figure 5). Within approximately four days the zoosporangium produces up to 300 zoospores, which are released into the environment via a discharge tube (Figure 6 and 7). The cycle is repeated once the zoospore finds a suitable substrate to settle on. Zoospores can settle on the same host or on a new host if available. It has been suggested that *B. dendrobatidis* may not be an obligate parasite; that is, it could live saprophytically (on dead tissue) or in other non-amphibian hosts (Davidson et al. 2003; Longcore et al. 1999)

### Feeding habits

Most of the information on *B. dendrobatidis* nutrient requirements is derived from laboratory studies aimed at optimizing chytrid culture conditions. Tryptone, gelatin hydrolysate, and lactose are used to culture *B. dendrobatidis* (Longcore et al. 1999; Piotrowski

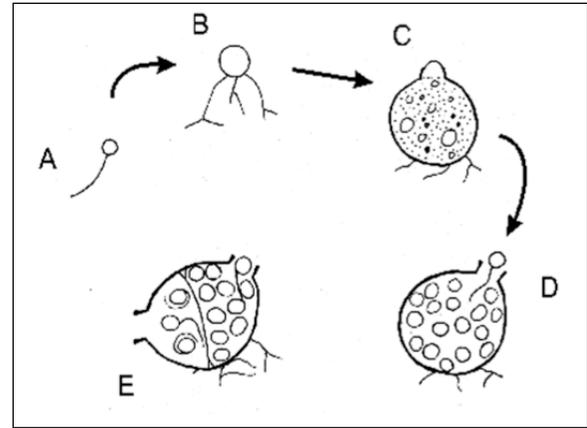


Figure 4. The amphibian chytrid life cycle. The life cycle of the fungus starts when the motile zoospore (A) attaches to the epidermal layer (B) of its host and penetrates the stratum corneum. Once attached, the zoospore transforms into a zoosporangium (C). The zoosporangium grows larger and more complex (D), and eventually produces additional zoospores (E). During development a discharge papilla is formed (C). This papilla allows the zoospores to escape the zoosporangium when the cap is lost (D). Source: Berger et al. 2005.

et al. 2004). Although *B. dendrobatidis* occurs only within keratinized cells of its host, it is uncertain if the fungus actually uses keratin as a nutrient (Piotrowski et al. 2004). Chytrid can be cultured in the absence of keratin.

### Reproduction

*B. dendrobatidis* is diploid and reproduces asexually, via aquatic unflagellated

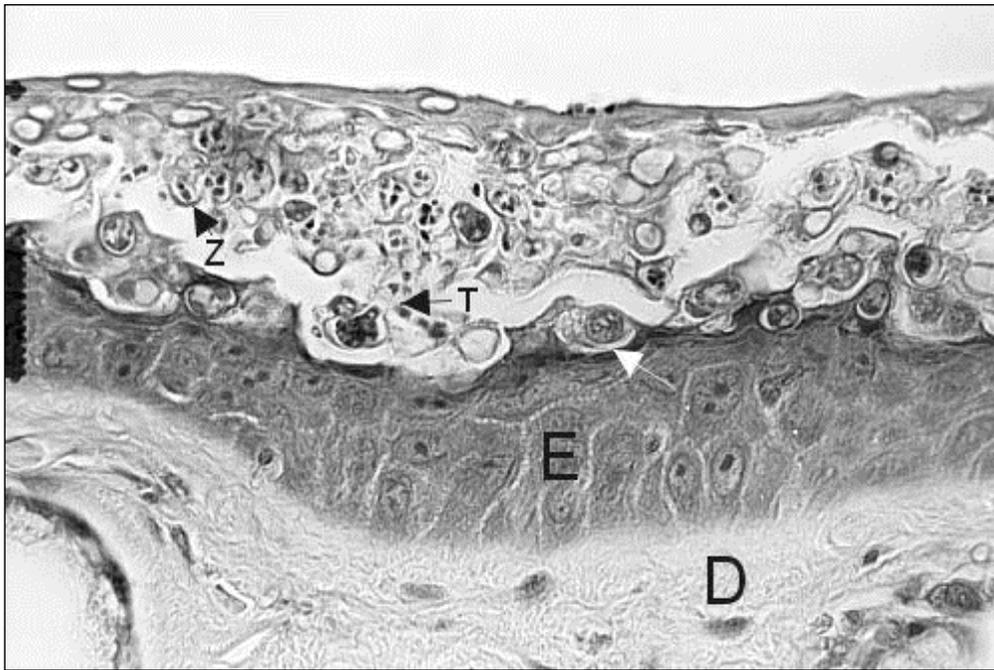


Figure 5. A photomicrograph of a section of skin from a frog infected with *B. dendrobatidis*. The zoosporangia of the fungus are the round structures in the stratum corneum. Zoospores develop within the zoosporangium (Z). Discharge papilla are evident deeper in the stratum corneum (T). The epithelial cells of the host respond to the presence of *B. dendrobatidis* by increasing in number. Normally a frog's stratum corneum is a layer about 2-3 cells thick. When infected the stratum corneum becomes multilayered and disorganized. E = epidermis, D = dermis. Source: Berger et al. 2005.

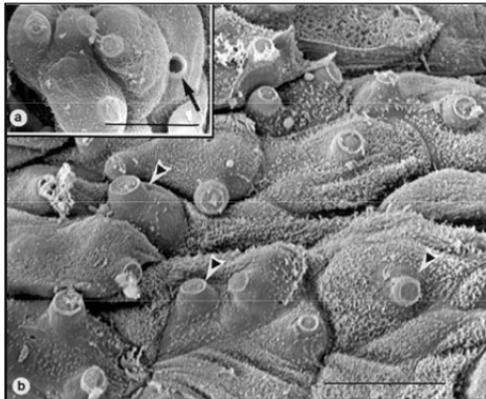


Figure 6. The epithelium of a frog infected with *B. dendrobatidis* showing the discharge papilla emerging from the surface. Normally the epithelial surface is relatively smooth and well organized. When infected the epithelium becomes roughened, the cells separate, and discharge papilla of *B. dendrobatidis* protrude out. Source: Berger et al. 2005.

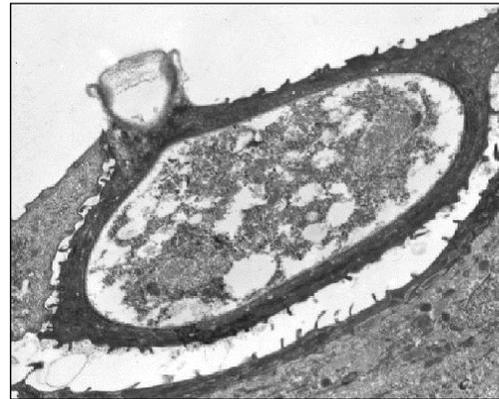


Figure 7. A cross section of a zoosporangium before its contents have organized into individual zoospores. A cap blocks the discharge papilla until the zoospores have completed their development. Once developed the plug is shed and the zoospores escape into the water or onto the skin surface to infect adjacent epithelial cells. Source: Berger et al. 2005.

zoospores that are produced within a zoosporangium (Johnson and Speare 2003). As of yet, no sexual stage has been observed for *B. dendrobatidis*. Genetic analysis of chytrid has shown populations to be mainly clonal (Morehouse et al. 2003). Other chytrid fungi lack a sexual stage.

No sexual or asexual resting structures have been identified in *B. dendrobatidis*. Some species of chytrid fungi possess a sexual stage, resulting in the production of thick-walled resistant resting spores.

#### *Environmental optima and tolerances*

*B. dendrobatidis* is found in a broad range of environments. Mortality resulting from chytridiomycosis occurs mainly at cooler times of the year for a given location, or in cool high altitude regions (Berger et al. 1999, Piotrowski et al. 2004). In culture chytrid grows from 4 to 25° C, but grows most rapidly from 17 to 25° C (Piotrowski et al. 2004). At temperatures  $\geq 28^\circ$  C, and below 10° C, chytrid growth ceases, or occurs slowly. Infections at these temperatures are not likely to cause immediate mortality since growth of fungus is not favored (Piotrowski et al. 2004).

According to Piotrowski et al. (2004), unless there are strains of chytrid with different temperature constraints, disease-related die-offs will be limited to cooler areas. In temperate areas, outbreaks may occur in montane areas in warmer months, or in the lowlands during the winter.

The pH optimum for *B. dendrobatidis* ranges from 6 to 7, conditions commonly found in freshwater systems. Chytrid grows poorly below pH 6, but it can survive, and once inside a host, *B. dendrobatidis* may be buffered from external conditions (Piotrowski et al. 2004). Other genera of chytrid fungi have similar temperature and pH tolerances.

*B. dendrobatidis* zoospores require water for transmission. They can survive for up to 7 weeks in sterile lake water (Johnson and Speare 2003), but cannot survive desiccation. Chytrid fungus experience 100% mortality within three hours of drying, and since no long term resting stage has been identified, it is not thought to persist for long periods outside of water in the zoospore stage. Thus, *B. dendrobatidis* is unlikely to persist in ephemeral waterbodies.

#### *Biotic associations*

*B. dendrobatidis* infects two amphibian orders, Anura (frogs), and Caudata (salamanders and newts), 14 families and at least 200 species (Hyatt et al. 2007). Of the anurans, it is more frequently associated with aquatic species than species with highly terrestrial adult stages or shorter larval periods (Pearl et al. 2007).

The zoospores of *B. dendrobatidis* rarely swim more than 2 cm prior to encysting, thus their distribution is greatly aided in flowing waterbodies (Kriger and Hero 2007). Given that chytrid fungus prefer cooler temperatures, they are more likely to grow in streams than ponds, since streams typically have lower temperatures.

This is consistent with observations that the most intense chytrid outbreaks occur in stream breeding species. Unlike terrestrial species, water-breeding amphibians tend to congregate at breeding sites, increasing the risk of becoming infected from diseased individuals. Kriger and Hero (2007) found few animals in ephemeral ponds with chytridiomycosis.

Completely terrestrial species of anurans (lacking an aquatic tadpole stage) have been observed with *B. dendrobatidis*, suggesting frog-to-frog transmission is possible (Kriger and Hero 2007). These are rare cases, and it is currently unclear how large of a role this type of transmission plays in the natural environment.

One of the enigmatic features of *B. dendrobatidis* is that it has little host selectivity (Hyatt et al. 2007). Thus, it doesn't depend on any single host species to persist. *B. dendrobatidis* also infects some amphibian species with little negative effects on the host. Invasive frog species such as the American bullfrog (*R. catesbeiana*) acquire chytridiomycosis, but do not die, and therefore may serve as reservoirs of the disease (Mazzoni et al. 2003).

## **Invasion Process**

*Current pathways, vectors, and routes of introduction*

*Global trade of amphibians for human consumption*

The trade of live amphibians for food may be an important pathway for the movement of chytrid fungus. One staple of this food trade, the American bullfrog (*R. catesbeiana*), is a known carrier of *B. dendrobatidis* that does not succumb to the disease. *R. catesbeiana* may be an important vector of the disease as populations from the food trade escape and become established (Mazzoni et al. 2003; Weldon et al. 2004).

Other *Rana spp.* and *Leptodactylus spp.* are traded internationally as food items for human consumption. These animals are produced in frog farms or captured from wild populations, with a rise in annual total production to 6,657 tons over the period from 1987 to 1998 (Table 1, Teixeira et al. 2001). Asian and Latin American countries are currently the greatest producers of amphibians for consumption. North American, European, and Asian countries are the largest consumers of these products. Between 1998 and 2002, the United States imported 777,309 live bullfrogs (Schlaepfer et al. 2005), and a total of 4 million amphibians for human consumption. If body parts and products are included in the accounting, the trade of amphibians for consumption becomes much larger (Schlaepfer et al. 2005). According to the OIE Aquatic Animal Health Standards Commission (2006), international trade in amphibians as food products will continue to grow, and without increasing animal health standards, there will be an inevitable increase in the associated distribution of amphibian infectious diseases.

Table 1. Production (in tons) of ranid frogs for human consumption, broken down by country. It should be noted this data is incomplete. For example, China, a major producer of frogs for the live food trade, is not included.

Source: Teixeira et al. 2001.

<i>Country</i>	<i>1987</i>	<i>1988</i>	<i>1989</i>	<i>1990</i>	<i>1991</i>	<i>1992</i>	<i>1993</i>	<i>1994</i>	<i>1995</i>	<i>1996</i>	<i>1997</i>	<i>1998</i>
<b>Argentina</b>	0	0	0	0	0	0	50	50	50	50	50	50
<b>Bangladesh</b>	2318	2824	2685	739	318	771	700	700	0	0	0	0
<b>Brazil</b>	10	29	40	60	90	120	140	165	304	415	522	570
<b>Cuba</b>	224	235	203	137	96	73	52	52	62	69	46	28
<b>India</b>	0	13	15	29	-	-	-	-	-	-	-	-
<b>Indonesia</b>	689	1582	1342	1590	1957	2666	2411	2111	2194	1793	1390	1330
<b>Mexico</b>	1327	1007	461	868	309	350	352	350	547	414	2063	1229
<b>Romania</b>	29	0	0	0	0	2	0	0	0	0	0	41
<b>Taiwan PC</b>	-	-	327	134	188	784	2052	1132	1378	1259	730	1700
<b>Thailand</b>	20	20	6	5	18	131	321	353	137	1600	1570	1600
<b>Turkey</b>	-	-	-	-	1321	648	750	851	864	740	160	100
<b>Uruguay</b>	0	1	0	-	-	-	-	-	15	3	3	3
<b>USA</b>	1	4	1	0	9	18	20	17	5	2	9	6
<b>TOTAL</b>	<b>4618</b>	<b>5715</b>	<b>5080</b>	<b>3562</b>	<b>4306</b>	<b>5563</b>	<b>6848</b>	<b>5781</b>	<b>5556</b>	<b>6345</b>	<b>6543</b>	<b>6657</b>

This OIE Commission estimates the global trade of live frogs for food to be 5 million animals per annum, and this is likely a gross underestimate. According to Lau et al. (1996) 6 million wild-caught edible frogs were imported to Hong Kong from Thailand over a one-year study period. The true number is probably in the tens of millions per year (OIE Aquatic Animal Health Standards Commission 2006).

#### *Global trade of amphibians by the pet industry*

The trade of diseased amphibians may also be an important pathway for the movement of chytrid globally. Although accurate data on the amphibian pet trade are not available, the number is at least 6 million individuals per year (OIE Aquatic Animal Health Standards

Commission 2006). Schlaepfer et al. (2005) found an average of 1 million individuals were shipped into the United States each year between 1998 and 2002. All of the reports of chytridiomycosis in Germany are for species shipped there as part of the pet trade, emphasizing the importance of this pathway for disseminating chytrid fungus.

#### *Global trade of amphibians by zoos*

Captive amphibians in zoos have also tested positive for *B. dendrobatidis* (Berger et al. 1999). Chytrid infected amphibians have also been found in European zoos (Daszak et al. 2000). If these animals are traded within the international zoo community, chytrid fungus will be further disseminated.

### *Global trade of amphibians for ornamental aquatic gardens*

Dwarf African clawed frogs (*Hymenochirus curtipes*) bred in captivity in California for ornamental aquatic gardens have tested positive for *B. dendrobatidis* (Groff 1991). The trade of this species in the United States in the 1980s may have facilitated the spread of chytrid fungus (Daszak et al. 1999)

### *Global trade of amphibians for use in laboratory studies*

Wild caught amphibians have been traded extensively for use in laboratory studies in the United States. African clawed frogs (particularly *X. laevis*) have been traded since 1934 for laboratory use. Initially used in pregnancy testing, *Xenopus spp.* has been more recently used in immunological studies (Weldon et al. 2004). From 1998 to 2004, an estimated 10,000 *X. laevis* were exported from South Africa. Captive bred and wild-caught *Xenopus spp.* are still traded internationally, and feral populations of *Xenopus* are established in at least four countries (OIE Aquatic Animal Health Standards Commission 2006).

### *Other vectors*

Additional vectors of chytrid movement include contaminated soil or water transported with other products in the pet trade, by wildlife such as birds (Johnson and Speare 2005), and by scientists when sampling fish, amphibians, or other aquatic organisms (Halliday 1998).

### *Factors influencing establishment and spread*

Factors influencing the establishment and spread of chytrid fungus are highly debated. Two of the best-documented cases of chytrid spread occurred in montane regions of Australia and Panama. In Australia a chytrid-related die-off wave progressed northward at 100 km per year (Pounds et al. 2006). Lips et al. (2006) documented a chytrid wave spreading along the high altitude regions of Central America, with movement occurring at approximately 30 km per year. Differences in ecological factors such as host population density, habitat, and age structure may influence the rate at which chytrid spreads through the environment (Daszak et al. 1999). The timing of introduction may also play a role in the rate of disease movement.

Chytrid fungus grows most rapidly at cool temperatures and many *B. dendrobatidis* associated die-offs occur during cooler seasons. Shifts in global climate may be favoring the spread of chytrid fungus, by shifting temperature regimes to those that favor the fungal growth (Pounds et al. 2006). Other researchers, such as Lips et al. (2006) refute this hypothesis, suggesting the disease moves as a classic epidemiological wave, without the need to invoke climate change as a causal agent.

The low host selectivity of chytrid greatly enhances its ability to spread to new populations. That is, the disease does not require a particular species of host, whose own range may limit the range of the disease. This is

one of the most enigmatic features of *B. dendrobatidis*, and may explain its global success and spread. Further, certain amphibian species carry the disease without showing any clinical signs or symptoms. These species probably act as reservoirs of the disease (Mazzoni et al. 2003). Otherwise the high virulence of the disease would likely cause it to burn out before moving across such broad swaths of land, as it has in Australia and Central American. These species include the American bullfrog, the cane toad, and the African clawed frog, all of which are also invasive. The spread of these reservoir species, both on their own, and via global trade pathways (see above) will likely continue to move chytrid into novel regions. Finally, chytrid may also exist saprophytically, which would allow it to persist after decimating host populations (Daszak et al. 1999).

The infectious agent of chytrid fungus is aquatic, as are the life histories of most of its amphibian hosts, so its spread will be related to the availability of aquatic habitat. Different species of amphibians utilize aquatic habitats for differing periods of time, and at different stages in their life history. More highly aquatic amphibians are most likely to be susceptible to the disease (Kriger and Hero 2007). The geographic range of a chytrid host will also determine the likelihood of chytrid spread, but many amphibians have highly restricted ranges and limited movement.

Some researchers have proposed that other environmental disturbance may influence the

spread of chytrid, mainly by depressing the immune system of amphibians. Such disturbance includes thinning of the ozone, and hence increased exposure to ultraviolet light, habitat destruction, or chemical pollution. Associations between chytridiomycosis and these disturbances have not yet been proven (Daszak et al. 1999).

#### *Potential ecological and economic impacts*

##### *Non-market impacts*

Chytrid disease is considered one of the greatest threats to vertebrate biodiversity in recorded history (Skerratt et al. 2007). Amphibian disease resulting from *B. dendrobatidis* is often fatal, and infection can result from as few as 100 zoospores (Daszak et al. 1999). Many amphibian populations already in decline due to habitat degradation may be completely eliminated when faced with the disease.

Chytrid is implicated in the catastrophic decline of many regional populations of frogs. It has driven some endemic species such as the golden toad (*Bufo periglenes*) in Costa Rica to extinction (Table 2, Daszak et al. 1999). Die-offs resulting from chytrid disease can occur over just a few months. In one study area, the introduction of chytrid fungus to amphibian populations resulted in a loss of half of the species and over 70 percent of the total numbers of frogs within four months (Lips et al. 2006). In Australia as many as seven amphibian species may have been driven to extinction due to

Table 2. Locations of some massive die-off resulting from chytridiomycosis. This table is incomplete, as additional chytrid-related die offs have been reported since 1999. Source: Daszak et al. 1999.

Locality and date of mass deaths	Species affected and impact <sup>a</sup>
E. & S. Australia (1993-1999) <sup>b</sup>	Multiple montane rain forest and temperate species. Mass deaths, local extinctions, population declines. Near-extinction of <i>Taudactylus acutirostris</i> . Hypothesized link with global extinction of two species of gastric brooding frog ( <i>Rheobatrachus</i> spp.).
W. Australia (1998-1999) <sup>c</sup>	Multiple species, predominantly the western green (or motorbike) frog ( <i>Litoria moorei</i> ). Mass deaths, population declines.
Costa Rica and Panama (1994-99)	Multiple montane rain forest species. Mass deaths, local extinctions, population declines. Hypothesized link with global extinction of golden toad, <i>Bufo periglenes</i> .
Ecuador (1999)	Montane rain forest <i>Atelopus</i> species, <i>Telmatobius niger</i> , and <i>Gastrothecus pseustes</i> . Unknown impact.
Arizona (1996-1997)	Leopard frog ( <i>Rana yavapiensis</i> & <i>R. chiricahuensis</i> ). Mass deaths.
S. Arizona (1999)	Leopard frog ( <i>Rana</i> sp.). Mass deaths.
Colorado (1999)	Boreal toad ( <i>Bufo boreas</i> ). Mass deaths.
Colorado (1970s)	Leopard frog ( <i>Rana pipiens</i> ). Mass deaths.
Sierra Nevada, California (1970s)	Yosemite toad ( <i>Bufo canorus</i> ). Mass deaths.

chytridiomycosis (Daszak et al. 1999). In the United States, chytridiomycosis may be linked to declines of the boreal toad (*Bufo boreas*) in Colorado, the Yosemite toad (*Bufo canorus*) and Mountain yellow-legged frog (*Rana muscosa*) in California, and the near extinction of the Wyoming toad (*Bufo baxteri*) in Wyoming (Pearl et al. 2007). Many die-offs resulting from the introduction of *B. dendrobatidis* are undocumented, due to remoteness of the regions where the disease flourishes

The greatest impacts are likely to occur for species that reside in environments that favor chytrid growth and transmission, such as regionally endemic rainforest specialists. These species are typically large bodied, have low

fecundity, and are mainly found at high altitudes and reproduce in streams (Daszak et al. 1999)

The total loss of amphibians in the most devastated zones will fundamentally alter the affected ecosystem. Frogs prey on insects such as mosquitoes, many of which are vectors of human disease. The larger frogs of South America eat rodents and so are an important component of pest control there. Amphibians, in turn, are the prey of snakes, birds, and mammals. Thus, the loss of amphibians will cause cascading alterations throughout the impacted ecosystem. Not all *B. dendrobatidis* infections result in die-offs. Some species can persist with the disease, or recover after population declines (Davidson et al. 2003; Retallick et al. 2004).

### *Market impacts*

Amphibians are traded internationally in the pet trade and for human consumption (see above). The loss of amphibian species resulting from chytrid disease will surely result in market losses in these trades. It is also likely the cost of trading these organisms will increase as regulatory scrutiny of these industries increases. Amphibians and their byproducts are also a major source of potential drugs for the pharmaceutical industry. Therefore, losses in amphibian diversity will reduce the potential pool of new drugs.

### **Management strategies and control methods**

In 2001 *B. dendrobatidis* was listed on the Office International des Epizooties (OIE) as a wildlife pathogen of paramount importance (Hyatt et al. 2007). This was the first time OIE listed an amphibian disease. The recommendations based on this listing are still being developed. In the mean time, OIE advises that amphibians shipped in international trade should be placed in a different container upon arrival to their destination, and that all water, soil, plants, and litter carried with the amphibian during shipping should be disinfected.

Management strategies to contain the spread of *B. dendrobatidis* include the detection of wild and captive populations infected with chytrid disease, identifying infected geographical areas, and controlling human-mediated movement of diseased animals from one location to another

(Hyatt et al. 2007). Trade routes of *B. dendrobatidis* reservoir species such as *X. laevis* exported from Africa to North America and Europe need to be explicitly identified (Weldon et al. 2002, OIE Aquatic Animal Health Standards Commission 2006), so they can be more adequately regulated.

Joe Mendelson of the Zoo Atlanta and Ron Gagliardo of the Atlanta Botanical Garden have developed emergency captive breeding programs for frogs threatened by the fungus (Mendelson et al. 2006). They hope to keep the most imperiled species alive until a method to clean the environment of chytrid fungus is discovered. Restocking the natural environment with chytrid-resistant amphibian genotypes is also being discussed, although methods of selecting for this resistance need to be developed (Australian Threat Abatement Plan 2006).

Although there is currently no method known to eradicate *B. dendrobatidis* once it is established (Kriger and Hero 2007), research programs are underway to determine mechanisms and evolution of chytrid resistance, control of the disease, and treatment of fungal-infected animals (Mendelson et al. 2006). No vaccine currently exists for chytridiomycosis prevention.

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### **Other useful sources of information**

Amphibian Research and Monitoring Initiative homepage. <http://armi.usgs.gov/>

Australian Threat Abatement Plan. Australian Government Department of Environment and Heritage; Canberra. 2006

<http://www.environment.gov.au/biodiversity/threatened/publications/tap/chytrid/index.html>

Dr. Rick Speare's Amphibian Diseases Homepage.

<http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm>

Fungi of Australia glossary. Compiled by C.A.Grgurinovic.

<http://www.anbg.gov.au/glossary/webpubl/fungloss.htm#operculate>

Global Amphibian Assessment (2004)

<http://www.globalamphibians.org/>

Global Invasive Species Database *B. dendrobatidis* page.

<http://www.issg.org/database/species/ecology.asp?si=123&fr=1&sts=>

### **Current research and management efforts**

#### *Global research initiatives*

The threat of *B. dendrobatidis* is considered so great that fifty of the leading researchers in the field recently called for the

formation of an Amphibian Survival Alliance, to be led by the World Conservation Union (IUCN). In the plan the authors request \$400 million for an initial 5-year budget to develop conservation programs to prevent further amphibian declines (Mendelson et al. 2006)

Many of the facets of *B. dendrobatidis* remain unknown, and the overall threat it poses is highly debated (McCallum 2005). Researchers are working to elucidate mechanisms of chytrid spread, both natural and human-assisted, and are trying to discern what other factors may enhance or alleviate the impact of the disease. Current research is also underway to understand the mechanism by which *B. dendrobatidis* kills amphibians, in hope that this information will aid in the treatment of infected individuals. Amphibians that appear immune to the disease or whose populations appear to recover after infection are also being studied to determine if genes for resistance are involved, or to identify mechanisms of host immunity defense (such as anti-microbial peptides).

Detailed knowledge of the global distribution of the disease is still unknown, and so many current research efforts are underway to document where chytrid resides, and what populations are still naïve to the disease. Current management efforts also include increasing the scrutiny of amphibian trade practices (see section 6 above).

#### *The Pacific Northwest*

Little is known about the distribution of chytrid disease in the Pacific Northwest.

Michael Adams, an ecologist at the USGS Forest and Rangeland Ecosystem Science Center in Corvallis, leads a team that is surveying sites for chytrid in California, Oregon, Washington, and Alaska as part of the Amphibian Research and Monitoring Initiative.

Andrew Blaustein, a professor of zoology at Oregon State University, and an expert in amphibian disease also studies chytrid disease in the Northwest. His team is testing whether environmental changes such as increased exposure to UV-B radiation, a bi-product of the earth's thinning ozone layer, affects amphibian susceptibility to the disease.

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