SEPARC
Information Sheet 2R

BATRACHOCHYTRIUM DENDROBATIDIS

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Amphibian Die-offs:

Infectious wildlife diseases have been emerging at an increasing rate[1]. One of these emerging infectious diseases, chytridiomycosis, threatens amphibian biodiversity – the most imperiled group of vertebrates[2]. Chytridiomycosis is caused by the aquatic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), which colonizes the keratinized skin of amphibians[3]. *Bd* has been detected on hundreds of amphibian species (see http://www.bd-maps.net for a detailed list of *Bd*-positive species and areas), suggesting the pathogen has a low host specificity[4]. Its effect on a population is dependent on host susceptibility, which differs among species. *Bd* has been detected in amphibian populations across the globe including North America[5–8], Central America[3,9], Australia[3], South America[10,11], Africa[12,13], and Europe[14–16]. Severe declines have occurred in areas not affected by habitat loss[14,17].

As for the southeastern United States, *Bd* has been detected in both frogs and salamanders in multiple states at relatively low prevalence rates (Table 1). The fact that *Bd* is widespread in this region and has a low occurrence suggests that the pathogen is endemic. However, the impact of *Bd* on amphibians in the Southeast has not yet been determined and is difficult to measure[18].

Pathogen Characteristics:

Chytridiomycosis was first observed in 1998 in amphibians of Australia and Central America[3] and the pathogen was later characterized in 1999[19]. *Bd* has two major stages during its life cycle: a zoospore stage characterized as the infective phase and a zoosporangium stage characterized as the growing phase[20]. The zoospore stage is the only part of the life cycle when the microorganism is motile[21]. Zoospores lack cell walls and are mostly spherical with a posterior flagellum that allows the organism to move[20,22]. After a period of motility, the zoospore encysts and becomes a germling (immature zoosporangium) as rhizoids develop[20,23]. The germling then develops and matures by increasing in cell mass[22]. Once mature, the zoosporangium produces flagellated zoospores[20]. During this process, discharge papillae form where zoospores will eventually leave the zoosporangium [22]. At maturity, plugs which block discharge papillae are dissolved and motile zoospores are released. Released zoospores are then able to reinfect the same individual or infect another amphibian [24].

Bd can grow under many conditions. The optimal temperature for *Bd* growth is between 17 and 25°C[23]. At temperatures above 28°C and below 10°C, the fungus grows very slowly or may stop growing completely[23]. If the body temperature of infected amphibians is raised above 37°C for a period of time, it can kill the pathogen[25]. The chytrid fungus can also tolerate a wide range of pH conditions. Growth of the fungus can occur between a pH of 5 and 10, with optimal pH between 6 and 7[23]. There is a lack of evidence for optimal moisture conditions; however, there is evidence that complete desiccation kills *Bd*[26]. Water is essential for the dispersal of *Bd*, which occurs during the zoospore stage of the life cycle[21].

Transmission:

Bd may spread from amphibian to amphibian during close contact[23,24]; this may include activities such as mating and during times when larvae school[23]. Experiments performed by Rachowicz and Vredenburg[24] demonstrated that

infected tadpoles could transmit the pathogen to non-infected tadpoles and to post-metamorphic amphibians. Studies also indicate that amphibians infected with the chytrid, but do not develop chytridiomycosis, can act as reservoirs such as the American bullfrog, *Lithobates catesbeiana*[24,26,27]. Transmission of *Bd* over large geographic areas is likely due to the global trade of amphibians for food, research, and pets[28–30] Additionally, human activities such as outdoor recreation and field research may be responsible for the spread of *Bd*[31].

Recent evidence suggest that other animals such as crayfish, nematodes, waterfowl, and reptiles may act as reservoirs[32–35].Water may also serve as a reservoir for the pathogen. For example, Johnson and Speare[26] demonstrate that *Bd* can survive and release zoospores in lake water in the lab for up to seven weeks. However, it is still uncertain whether *Bd* can grow and persist on non-amphibian hosts and substrates in nature.

Signs of Disease and Diagnostic Testing:

Susceptibility to chytridiomycosis is species specific and may vary within a species. Infection begins with the colonization of the keratinized cells of the amphibian's epidermis[22]. Infections most often occur on the ventral surface of the amphibian, but may occur anywhere on the skin[19]. During infection, an amphibian may undergo physical and behavioral changes. Hyperkeratosis, which is characterized as an increase in the thickness of the skin (stratum corneum) due to hyperplasia, is most often seen in infected amphibians. Infected individuals may experience an increase in skin thickness of two to five times that of healthy individuals[3,20,22]. Excessive sloughing of skin is also associated with the disease[3]. If sloughing occurs faster than an amphibian can regenerate new skin, it may lead to exposure of non-keratinized skin[20]. Lesions have been found on some infected individuals[19]. Signs of infection may also include behavioral changes, which vary among individuals. Anorexia, lethargy, and unresponsiveness to stimuli are among the most common behavioral changes [21].

Mortality can occur in many species and frequently takes place between 18 and 48 days after infection[3,6,8,36–38]. Strong evidence suggests that death results from impairment to regulatory function of the skin. This damage disrupts the exchange of electrolytes across the skin resulting in cardiac arrest[39].

Diagnostic testing for *Bd* includes histological examination and molecular tests (PCR or real-time PCR). A detailed summary and comparison between histological methods and real-time PCR can be found in Kriger et al.[40]. A detailed protocol of using PCR to assay for the presence of *Bd* is given in Annis et al.[41]. Real-time PCR methodology is provided in Boyle et al.[42] and Hyatt et al.[43]. A real-time PCR protocol to test for the presence of *Bd* in soil and water samples is given in Kirshtein et al.[44]. Kosch and Summers[45] provide a good protocol to limit the amount of inhibitors that negatively affect *Bd* PCR.

Factors Contributing to Emergence:

The origins of *Bd* are currently unclear and there is evidence to suggest that *Bd* is not a new disease to North American amphibian populations. Amphibian specimens from several time periods were sampled for infection and the earliest reliable cases were dated to 1961 found in *Rana clamitans*. The sampling for the time period 1960-1969 was a total of 655 specimens, 46 of which were infected [7]. This is an indication that *Bd* has been present within amphibian populations in North America as far back as the 1960's and possibly earlier[7].

Other current hypotheses allude to commercial trade and globalization being one of the largest factors in the prevalence of *Bd* today. Specimens of *Xenopus laevis* from 1938 are the earliest samples found to be infected currently[13]. *X. laevis* appears to only act as a carrier[13,46]. This species became important to the medical community in 1934 when findings showed that they were useful as pregnancy assays for humans and their mass globalization could have led to infection of naïve amphibian populations as escaped and released frogs developed wild populations[13,47,48]. The global trade of *Lithobates catesbeiana* as well as other amphibians may also have played a large role in the dispersal of *Bd*[29].

Recent genetic research has provided more evidence for the hypothesis that *Bd* is a novel pathogen rather than one that has been endemic to populations for long periods of time. James et al.[49] found through genetic sequencing that the current pathogen is the result of a severe genetic bottleneck which resulted in a single diploid lineage with only two alleles per locus. Since *Bd* has several adaptations to living in the skin, the current hypothesis states that there was likely a hybridization that increased the virulence of the fungus[49]. The work of Joneson et al.[50] supports this hypothesis through their discovery of differences between *Bd* and its closest relative *Homolaphlyctis polyrhiza*. The genome of *Bd* was found to contain many more specific genes for metalloproteases, serine-type proteases, and aspartyl proteases,

gene families which have been seen in other fungal vertebrate pathogens. This unique set of genes, although not new to the fungus, may have had devastating effects when hybridized with another strain.

The hypothesis regarding hybridization is further supported by globalization through the pet trade. Farrer et al.[51] have found three distinct lineages of *Bd* which are referred to as "Global Panzootic, Swiss, and Cape lineages." The global lineage's genome shows unique traces of recombination as well as differing morphological traits when compared to the other strains which have been isolated allopatrically. Circumstantial evidence suggests that the Cape and Swiss lineages are hypovirulent, as they have not been linked to any specific population declines in their current regions. However, the Global Panzootic lineage is hypervirulent and has been found on five continents in areas where major amphibian declines have occured. Farrer et al.[51] predicted that the emergence of this lineage occurred between 35 and 257 years before present.

Conservation Strategies:

Strategies to prevent outbreaks or the spread of the disease are difficult due to the possible presence of the pathogen in animal and environmental reservoirs[26,27,32–35]. Currently, the only conservation effort being implemented to prevent species from extinction is placing highly threatened species in survival assurance colonies[52,53].

Other conservation strategies focus on preventing the spread of *Bd* to uninfected amphibian populations and controlling the disease in captive colonies[53]. Phillott et al.[54] provide detailed disinfection protocols to prevent the spread of *Bd* within and between populations. Protocols to clear amphibians of infection with treatments of the antifungal chemical itraconazole[53,55–58]; however, there may be harmful side effects associated with treatment[55].

Recently, there has been an effort to develop strategies manage chytridiomycosis in nature[59]. Some of these strategies include immunization, reducing the density of susceptible amphibians, reintroducing amphibians that have been artificially selected to resist *Bd*, and controlling *Bd* populations with competitors or predators of *Bd,* such as zooplankton[59]. Evidence has shown that bio-augmentation (manipulation of the bacterial community on skin of an amphibian) may be a promising method to control and manage chytridiomycosis in nature[60–62]. Treatment of susceptible amphibian species with antifungal skin bacteria may allow re-introduced amphibians to co-exist with *Bd* in native habitats. Amphibians as a group are facing large population declines and extinctions due to chytridiomycosis, which emphasizes the need for research into management and prevention of this disease.

Table 1. *Bd* detection in the southeastern United States. States are bolded if *Bd* has been detected in the state.

Species	State	Infected (N)	Total (N)	Reference
Anurans				
Acris crepitans	GA, NC, SC, **VA**	2	39	63–66
Acris gryllus	FL, GA, SC	0	42	9,65,66
Anaxyrus americanus	GA, NC	0	13	9,63,66
Anaxyrus fowleri	GA, LA, **TN**, VA	11	197	9,64,66,67
Anaxyrus quercicus	FL, SC	0	2	9,65
Anaxyrus terrestris	GA, NC, SC	0	29	9,65,66
Bufo woodhousei	NC	0	2	63
Eleutherodactylus planirostris	FL	0	4	9
Gastrophryne carolinensis	GA, NC, SC	0	7	63,65,66
Hyla chrysoscelis	GA, NC, SC	0	12	63,65,66
Hyla cinerea	GA, FL, LA, NC, SC	0	283	9,63,65,68
Hyla femoralis	FL, SC	0	10	9
Hyla gratiosa	FL, SC	0	15	9,65
Hyla squirella	FL	0	28	9
Incilius nebulifer	LA	0	10	9
Lithobates capito	FL, GA	0	21	9,66,69
Lithobates catesbeiana	FL, **GA**, LA, MS, **NC**, **SC**, **VA**	86	387	9,63–66,69–71
Lithobates clamitans	FL, **GA, LA, NC**, SC	9	111	9,63,65,66
Lithobates grylio	FL, SC	0	20	9,65

Lithobates heckscheri	GA	0	1	[66]
Lithobates palustris	**GA**, NC, **VA**	4	25	[63,64,66]
Lithobates sphenocephala	**AR**, FL, **GA**, **LA**, MS, **NC**, **SC, TN**	18	408	[9,63,65,66,71,72]
Lithobates sylvatica	**GA**, NC, **TN**	5	128	[9,63,66,73]
Lithobates virgatipes	SC	0	1	[65]
Osteopilus septentrionalis	FL	0	5	[9]
Pseudacris crucifer	GA, LA, NC, SC, TN, **VA**	10	86	[9,63–66]
Pseudacris feriarum	GA, TN	0	5	[9]
Pseudacris fouquettei	**LA**	4	34	[9]
Pseudacris ornata	GA	0	1	[66]
Pseudacris triseriata	NC	0	1	[63]
Scaphiopus holbrookii	GA, SC	0	21	[65,66]
Caudates				
Ambystoma maculatum	GA, TN	0	46	[9,66]
Ambystoma opacum	GA, NC, TN	0	9	[9,63,66]
Ambystoma talpoideum	GA, MS	0	9	[9,66]
Ambystoma tigrinum	GA, NC	0	17	[9,63]
Amphiuma means	GA, **FL**, **MS**	17	38	[66,74]
Amphiuma pholeter	FL	0	1	[74]
Amphiuma tridactylum	**LA**	2	11	[74]
Cryptobranchus alleganiensis	**GA**, **MS**, NC, **TN**, WV	47	356	[75–78]
Desmognathus aeneus	GA	0	3	[66]
Desmognathus apalachicolae	GA	0	29	[66]
Desmognathus conanti	GA	2	86	[66]
Desmognathus fuscus	AL, **MD**, VA	1	28	[79–81]
Desmognathus imitator	TN	0	19	[63]
Desmognathus marmoratus	GA	0	7	[66]
Desmognathus monticola	GA, MD, NC, TN, **VA**	3	234	[9,63,66,80–82]
Desmognathus ochrophaeus	TN	0	3	[80]
Desmognathus ocoee	GA, **NC**	4	181	[66,82,83]
Desmognathus orestes	NC	0	20	[63]
Desmognathus quadramaculatus	GA, NC, TN	0	198	[9,63,66,80,82]
Desmognathus welteri	WV	0	20	[75]
Desmognathus wrighti	NC	0	1	[63]
Eurycea bislineata	NC, VA	0	15	[63,81]
Eurycea chamberlaini	GA	0	1	[66]
Eurycea cirrigera	GA, **AL**	21	97	[66,79]
Eurycea guttolineata	GA, AL	0	18	[66,79]
Eurycea lucifuga	GA, WV	0	61	[66,75]
Eurycea quadridigitata	GA	0	12	[66]
Eurycea wilderae	GA, NC	0	77	[66,82]
Gyrinophilus porphyriticus	AL, GA, NC, TN, VA, WV	0	28	[9,66,75,79–82]
Gyrinophilus subterraneus	WV	0	8	[75]
Hemidactylium scutatum	GA	0	1	[66]
Necturus alabamensis	**FL**	3	15	[74]
Necturus beyeri	GA, **LA**	1	3	[66,74]
Notophthalmus	NC	0	5	[63]

viridescens dorsalis				
Notophthalmus viridescens viridescens	**GA**, LA, **NC**, **TN**, **VA**	19	115	9,66,73
Plethodon cinereus	VA	0	142	81
Plethodon cylindraceus	VA	0	19	81
Plethodon glutinosus	AL, GA, **NC**	1	63	63,66,79
Plethodon metcalfi	NC	0	56	9,63
Plethodon nettingi	WV	0	43	75
Plethodon punctatus	WV	0	38	75
Plethodon richmondi	NC	0	6	63
Plethodon serratus	GA, NC	0	3	9,66
Plethodon shermani	**NC**	4	66	82,83
Plethodon ventralis	GA	0	6	66
Plethodon websteri	GA	0	2	66
Plethodon welleri	NC	0	4	63
Plethodon yonahlossee	**NC**	1	40	63
Pseudobranchus axanthus	**FL**	1	13	74
Pseudobranchus striatus	**FL**	0	1	74
Pseudotriton montanus	GA	0	4	66
Pseudotriton ruber	AL, GA, VA	0	22	66,79,81
Siren intermedia	**LA**, **MS**	2	5	74
Siren lacertina	**FL**	3	7	74

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