Occurrence of *Batrachochytrium dendrobatidis* in Amphibian Populations of Okomu National Park, Nigeria

Amphibian declines are frequently due to a complex array of causative agents, many of which are poorly understood (Kiesecker et al. 2001; Garner et al. 2006). Although habitat destruction is the primary driver of biodiversity loss, infectious diseases have been identified as an important cause of some of the observed amphibian declines and mass mortalities (Skerratt et al. 2007). In particular chytridiomycosis caused by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has been associated with amphibian mass mortality events throughout the world (Berger et. al. 1998; Bosch et. al 2001; Lips 1998).

A number of surveys have established the occurrence of chytridiomycosis on the African continent, including South Africa (Hopkins and Channing 2003; Lane et al. 2003), Lesotho and Swaziland (Weldon 2005), Kenya (Kielgast et al. 2009), Tanzania (Moyer and Weldon 2006, Weldon and du Preez 2004), Uganda (Goldberg et al. 2007), Democratic Republic of Congo (Greenbaum et al. 2008), Ghana (Morgan et al. 2007), Gabon (Bell et al. 2011; Da Versa et al. 2011; Gratwicke et al. 2011) and Morocco (El Mouden et al. 2011). All of these records with the exception of those from Ghana are from wild caught animals. Some of the countries that represent the distribution gaps in sub-Saharan Africa (e.g., Guinea, Ivory Coast, Central African Republic) and Madagascar have been surveyed to some extent, but no evidence of *Bd* was found (Weldon 2005; Weldon et al. 2008). Furthermore, with the exception of Madagascar, small sample sizes restrict the reliability of the data from these surveys (e.g., Skerratt et al. 2008). Despite its prominence in Africa, chytridiomycosis has been associated with the extinction in the wild of only one species, the Kihansi Spray Toad from Tanzania (Weldon and du Preez 2004; Krajick 2006), although habitat alteration also was implicated in that species' decline (Krajick 2006). Contrary to the catastrophic host response in this east African case study, epidemiological evidence from histopathological studies indicates an endemic infection in southern Africa (Weldon et al. 2004).

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Due to disparities in host response to chytridiomycosis in sub-Saharan Africa, a high degree of uncertainty exists with regards to how species from currently unsurveyed regions will respond to infection. Here we report on a *Bd* survey that was conducted in Okomu National Park, Nigeria between April 2007 and July 2008. Our objectives were to assess *Bd* occurrence in all amphibian species captured during a broad census of Okomu National Park. We also investigated whether any prevalence differences existed among amphibian families, sexes, and seasons. We hypothesized that males would have higher prevalence than females in aquatic-breeding species, because they are resident in water bodies for longer time periods in their search for mates. We expected higher prevalence to occur in the wet season rather than the dry season due to *Bd* being an aquatic-dependent fungus. We also expected similar prevalence among members of the same family due to their ecological similarities which predispose them to comparative *Bd* exposure.

Okomu National Park is situated within the largest block of what is now left of Guinea-Congo lowland rainforest in southwestern Nigeria and is characterized by a variety of habitats from swamp-forest to open scrub, making it ideal for amphibian diversity. It is situated between North latitudes 6°15' and 6°25', and East longitudes 5°9' and 5°23' (Fig. 1).

Frogs were captured monthly from April 2007 to July 2008 for a total of five dry season months and 10 rainy season months. Samples were collected by swabbing sterile cotton swabs five times over the ventral surface of the feet, thighs and body. Swab tips were stored in 2-ml screw-cap vials containing 1 ml of 70% ethanol. The samples were tested with TaqMan real time Polymerase Chain Reaction for the presence of amphibian chytrid fungus (Boyle et al. 2004). Unpaired t-test assuming equal variance was used to determine significant difference in overall prevalence of *Bd* between the two treatments wet and dry seasons and overall prevalence between the male and female treatment groups. Thus *Bd* is the response variable, and the combined species the experimental units. Chi-square test was used to test for significant difference in chytrid infection for each species (experimental units) between the wet and dry seasons and for each species between males and females.

A total of 732 frogs representing 10 families were sampled over the 15 month period. Of these, 7 families, 8 genera and 21 species were infected with *Bd* (Table 1). Members of the families Bufonidae, Dicroglossidae and Hemisotidae tested negative for *Bd*. An overall prevalence of 43.6% was recorded for infected species at Okomu National Park. The majority of frogs were collected during the wet season, which had an infection prevalence of 44%. Exceptionally high prevalence was detected for members of the Hyperoliidae (*Afrixalus nigeriensis*, up to 83%; *Silurana tropicalis*, 69%; and *Chiromantis rufescens*, 62%). Overall infection during the dry season was significantly lower at 33% (p < 0.05).

For the majority of species, more males than females were infected with *Bd* (Table 1). These differences between infected sexes were very particularly pronounced for *Afrixalus dorsalis* (100% males vs. 26.1% females infected, p < 0.001), *Hyperolius*

sylvaticus (86.6% males vs. 14.2% females infected, p < 0.001) and *Hyperolius* sp. 1 (40% males vs. 14.2% females infected, p < 0.001). Unpaired t-test confirmed that overall males were significantly more infected than females (p < 0.001). Generally, no clinical signs of chytridiomycosis could be observed except in *Chiromantis rufescens*, which exhibited excessive skin sloughing in 20% of animals. Microscopic examination of skin sloughs confirmed the presence of *Bd* thalli.

Although restricted to a small geographic area, our survey at Okomu National Park included a large enough sample size to make meaningful deductions. We confirm the presence of *Bd* in Nigeria, supporting an initial anecdotal report of infected *Chiromantis rufescens* (Imasuen et al. 2009). The level of infection that we detected in southwest Nigeria is among the highest that has been recorded anywhere in Africa. High infection levels in hyperolid frogs are consistent with previous findings in Tanzania (Moyer and Weldon 2006) and Kenya (Kielgast et al. 2009). However, large variation in *Bd* prevalence among members of the Hyperoliidae suggest that susceptibility to infection in this family is not only due to taxonomic relatedness, but that host-specific factors, such as behavior or reproductive mode, may be involved (e.g., Lips et al. 2003; Rowley and Alford 2007).

Two distinct patterns emerged from this study: heightened susceptibility to infection in the wet season and higher susceptibility to infection in males. In contrast to a peak in infection levels during the wet season, it has been shown that a cool climate (average summer maximum < 30°C) is the most significant factor for the presence of *Bd* in Australia, as opposed to rainfall or altitude (Drew et al. 2006). However, Collins et al. (2003)

demonstrated that synchronization of optimal temperature and hydric cycles influence *Bd* growth. Clearly no single model exists to predict *Bd* seasonal infection patterns in populations from different geographical regions. Gender has been identified as a
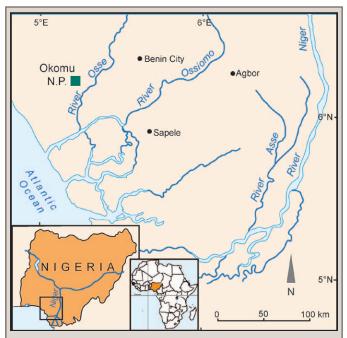

Fig. 1. Location of *Batrachochytrium dendrobatidis* sampling at Okomu National Park, Nigeria.

Table 1. Seasonal and gender infection levels of *Batrachochytrium dendrobatidis* (*Bd*) at Okomu National Park, Nigeria.

Family and Species	No. frogs collected (% *Bd* infected)			
	Wet Season	Dry Season	Males	Females
ARTHROLEPTIDAE				
Leptopelis hyloides	17 (47.0)	13 (00.0)	21 (33.3)	9 (11.1)
Leptopelis spiritusnoctis	10 (20.0)	7 (00.0)	10 (10.0)	7 (14.3)
HYPEROLIIDAE				
Hyperolius concolor	4 (50.0)	—	3 (33.3)	1 (00.0)
Hyperolius fusciventris burtoni	9 (33.3)	6 (16.6)	9 (44.4)	6 (00.0)
Hyperolius picturatus	9 (22.2)	12 (33.3)	14 (42.8)	7 (00.0)
Hyperolius sylvaticus	18 (66.2)	4 (50.0)	15 (86.6)	7 (14.2)
Hyperolius sp. 1	10 (10.0)	7 (57.1)	10 (40.0)	7 (14.2)
Hyperolius sp. 2	5 (40.0)	4 (00.0)	6 (33.3)	3 (00.0)
Hyperolius sp. 3	3 (00.0)	2 (50.0)	4 (25.0)	1 (00.0)
Hyperolius sp. 4	5 (40.0)	—	3 (66.2)	2 (00.0)
Afrixalus dorsalis	82 (75.6)	26 (46.1)	62 (100)	46 (26.1)
Afrixalus nigeriensis	42 (83.3)	—	35 (94.3)	7 (28.5)
Afrixalus paradorsalis	6 (66.6)	—	5 (80.0)	1 (0.00)
PHRYNOBATRACHIDAE				
Phrynobatrachus calcaratus	22 (54.5)	3 (00.0)	18 (38.9)	7 (71.4)
Phrynobatrachus liberiensis	9 (44.4)	16 (50.0)	20 (40.0)	5 (0.00)
Phrynobatrachus plicatus	22 (27.2)	11 (00.0)	21 (19.0)	12 (16.6)
PIPIDAE				
Silurana tropicalis	100 (65.0)	42 (54.7)	78 (78.2)	64 (57.8)
PTYCHADENIDAE				
Ptychadena longirotris	27 (3.7)	—	23 (4.3)	4 (00.0)
Ptychadena pumilio	5 (00.0)	30 (20.0)	17 (11.7)	18 (16.6)
RANIDAE				
Amnirana albolabris	6 (33.3)	5 (20.0)	7 (42.6)	4 (50.0)
RHACOPHORIDAE				
Chiromantis rufescens	60 (61.7)	4 (75.0)	38 (68.4)	26 (53.8)

factor that influences disease expression in humans and animals due to differences in behavior and physiology (Mohamed-Ali et al. 1999; Liesenfeld et al. 2001). In frogs, such behavioral differences are pronounced in species that make use of a prolonged breeding strategy where reproductive activity may range from a few weeks to several months. Since female arrival at breeding sites occur at variable intervals, males invest their energy in the establishment and defense of stationary call sites, resulting in large aggregations of calling males (Wells 1977). Choruses are characterized by a high turnover of individual males, because vocalization is energetically expensive and males regularly need to replenish energy reserves (Passmore and Carruthers 1995). It is therefore plausible that general differences in gender infection levels could be as a result of males congregating for longer at *Bd*-infected sites. Our understanding of the disease dynamics of chytridiomycosis in Africa remains limited.

The lack of comprehensive distribution data for *Bd* on this continent, together with the unpredictability of how amphibian species from different climatic regions react to *Bd*, as well as quantitative data on how disease transmission is shared among sexes of a species, warrants the need for comprehensive field surveillance.

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ART IN HERPETOLOGY

The unpublished watercolor drawing of a Western Pond Turtle (*Actinemys marmorata*) figured here is one of more than 900 scientific illustrations in the Ernst Mayr Library's Jacques Burkhardt Collection, held at the Museum of Comparative Zoology (MCZ), Harvard University. This undated scientific illustration was drawn by artist Jacques Burkhardt, the personal and principal artist of MCZ founder Jean Louis Rodolphe Agassiz. Agassiz had commissioned such drawings in support of his seminal endeavor, *Contributions to the Natural History of the United States of America*, an anticipated 10-volume set of which only four volumes were realized. Lithographer A. (Auguste) Sonrel successfully adapted many of Burkhardt's turtle drawings for Agassiz's *Contributions* (vol. I: North American Testudinata, and vol. II: Embryology of the Turtle); however, this drawing of an adult specimen from San Francisco, California, was not selected for publication in Agassiz's failed masterpiece. The manuscript annotation penciled by Agassiz himself reads, "To be compared with *Emys nigra* Hallowell; from Lower California. This is from San Francisco." Agassiz's annotation no doubt refers to a second unpublished Burkhardt drawing of an adult Western Pond Turtle specimen collected in Southern California by naturalist Spencer Fullerton Baird. Additional information about the Jacques Burkhardt Collection can be found at: http://library.mcz.harvard.edu/. Special thanks to MCZ Director James Hanken for permitting reproduction of Burkhardt's work here.

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