TABLE 1. Anurans that tested positive or negative for the presence of *Batrachochytrium dendrobatidis* in Denmark in 2007. See Fig. 1 for the locations of the study areas referenced in the table. The species examined were *Rana* kl. *esculenta* (RANESC), and *Rana temporaria* (RANTEM).

Study Area	Latitude	Longitude	Species	Stage	Sex	No. Positive	No. Negative
Egense	55.044167	10.519444	RANTEM	adult	unknown	1	3
Amtoft	57.007500	8.939722	RANTEM	adult	male	0	1
Klosterheden	56.485278	8.362500	RANTEM	juvenile	unknown	0	1
Vestamager	55.614722	12.577222	RANESC	adult	male	0	3
Vestamager	55.614722	12.577222	RANESC	adult	female	0	1
Vestamager	55.614722	12.577222	RANESC	juvenile	unknown	1	2

Further surveys should be undertaken to determine the extent of the pathogen in Denmark. In the meantime, proper sanitizing of equipment would be prudent for anyone entering amphibian habitats. Particular care should be used around sensitive species such as *Bombina bombina*, which is actively managed in Denmark (Pihl et al. 2001), however the threat posed by *B. dendrobatidis* to this and other Danish species is currently unknown.

Acknowledgments.—These surveys were authorized by The Danish Forest and Nature Agency of the Ministry of the Environment with letter of 15 January 2008 (SNS-441-00088). No chemicals or other substances were used on the body of the amphibians and all criteria for the human care of captured animals were followed. Use of trade names does not constitute endorsement.

LITERATURE CITED

ANNIS, S.L., F.P. DASTOOR, H. ZIEL, P. DASZAK, AND J.E. LONGCORE. 2004. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. J. Wild. Dis. 40:420–428.

BERGER, L., R. SPEARE, P. DASZAK, D. E. GREEN, A. A. CUNNINGHAM, C. L. GOGGIN, R. SLOCOMBE, M. A. RAGAN, A. D. HYATT, K. R. MCDONALD, H. B. HINES, K. R. LIPS, G. MARANTELLI, AND H. PARKES. 1998. Chytridiomycosis causes amphibian mortality associated with population decline in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. USA 95:9031–9036.

CUNNINGHAM, A.A., T.W.J. GARNER, V. ANGUILAR-SANCHEZ, B. BANKS, J. FOSTER, A.W. SAINSBURY, M. PERKINS, S.F. WALKER, A.D. HYATT, AND M.C. FISHER. 2005. The emergence of amphibian chytridiomycosis in Britain. Vet. Rec. 157: 386–387.

GARNER, T.W.J., M. PERKINS, P. GOVINDARAJULU, D. SEGLIE, S.J. WALKER, A.A. CUNNINGHAM, AND M.C. FISHER. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biol. Lett. 2:455–459.

———, S. WALKER, J. BOSCH, A. D. HYATT, A. A. CUNNINGHAM, AND M. C. FISHER. 2005. Chytrid fungus in Europe. Emerg. Infect. Dis. 11:1639–1641.

MUTSCHMANN, F., L. BERGER, P. ZWART, AND C. GAEDICKE. 2000. Chytridiomycosis in amphibians—first report in Europe. Berl. Munch. Tierarztl. Wochenschr. 113:380–383.

OUELLET, M., I. MIKAELIAN, B. D. PAULI, J. RODRIGUE, AND D. M. GREEN. 2005. Historical evidence of widespread chytrid infection in North American amphibian populations. Conserv. Biol. 19:1431–1440.

PIHL, S., R., EJRNÆS, B. SØGAARD, E. AUDE, K.E. NIELSEN, K. DAHL. AND J.S. LAURSEN. 2001. Habitats and species covered by the EEC Habitats Directive: a preliminary assessment of distribution and conservation status in Denmark. NERI Technical Report No. 365. National Environmental Research Institute, Denmark. 121 pp.

SIMONCELLI, F., A. FAGOTTI, R. DALL'OLIO, D. VAGNETTI, R. PASCOLINI, AND I. DI ROSA. 2005. Evidence of *Batrachochytrium dendrobatidis* infection in water frogs of the *Rana esculenta* complex in central Italy. EcoHealth 2:307–312.

SKERRATT, L. F., L. BERGER, R. SPEARE, S. CASHINS, K. R. MCDONALD, A. D. PHILLOTT, H. B. HINES, AND N. KENYON. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

STAGNI G., R. DALL'OLIO, U. FUSINI, S. MAZZOTTI, C. SCOCCIANTI, AND A. SERRA. 2004. Declining populations of Apennine yellow-bellied toad *Bombina pachypus* in northern Apennines, Italy: is *Batrachochytrium dendrobatidis* the main cause? Ital. J. Zool. Suppl. 2:151–154.

STUART, S.N., J.S. CHANSON, N.A. COX, B.E. YOUNG, A.S.L. RODRIGUES, D.L. FISCHMAN, AND R.W. WALTER. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

Herpetological Review, 2008, 39(2), 200–202.

Batrachochytrium dendrobatidis Not Detected in *Oophaga pumilio* on Bastimentos Island, Panama

CORINNE L. RICHARDS*
AMANDA J. ZELLMER
and
LISA M. MARTENS
Department of Ecology and Evolutionary Biology
and
Museum of Zoology, University of Michigan, 1109 Geddes Avenue
Ann Arbor, Michigan 48109-1079, USA

**Corresponding author; e-mail: clrichar@umich.edu*

Amphibian chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), has been implicated in the decline and extinction of many populations worldwide (Berger et al. 1998; Lips et al. 2006; Skerratt et al. 2007), and has led to massive die-offs in Latin America over the past few decades (Lips et al. 2006). However, not all populations that carry *Bd* have experienced such declines (e.g., American Bullfrog: Garner et al. 2006; African Clawed Frog: Weldon et al. 2004). Some species have been shown to have physiological (Woodhams et al. 2007a), behavioral (C.L. Richards, pers. comm.), or bacterial (Woodhams et al. 2007b) defenses that may allow them to cope with *Bd*. For many species that have not experienced declines, it is unknown

whether they have lower susceptibility, due to such defenses, or if they have not been exposed to the disease. Evaluation of these alternatives is necessary for distinguishing between species that may be at risk of population declines and those that may be *Bd* carriers.

Despite the fact that an epidemic wave of *Bd* has apparently swept through Costa Rica and western Panama since the late 1980s (Lips et al. 2006), populations of the Strawberry Dart Frog, *Oophaga pumilio*, remain relatively stable. *Oophaga pumilio* is a dendrobatid frog found in rainforests of the Caribbean coast from Nicaragua to Panama. They are found across a range of elevations, from sea level to about 1000 m (Walls 1994). Populations of *O. pumilio* in the Bocas del Toro archipelago in Panama remain abundant and apparently healthy, and we are unaware of any chytrid-related die-offs in other areas of their range or any studies that have assessed *Bd* prevalence in this species. The stability of the Bocas del Toro populations suggests that either *O. pumilio* is able to physiologically or behaviorally cope with the disease or else *Bd* is not present in this region. To distinguish between these alternative hypotheses, we tested for the presence of *Bd* in *O. pumilio* across Bastimentos Island in the Bocas del Toro archipelago, Panama.

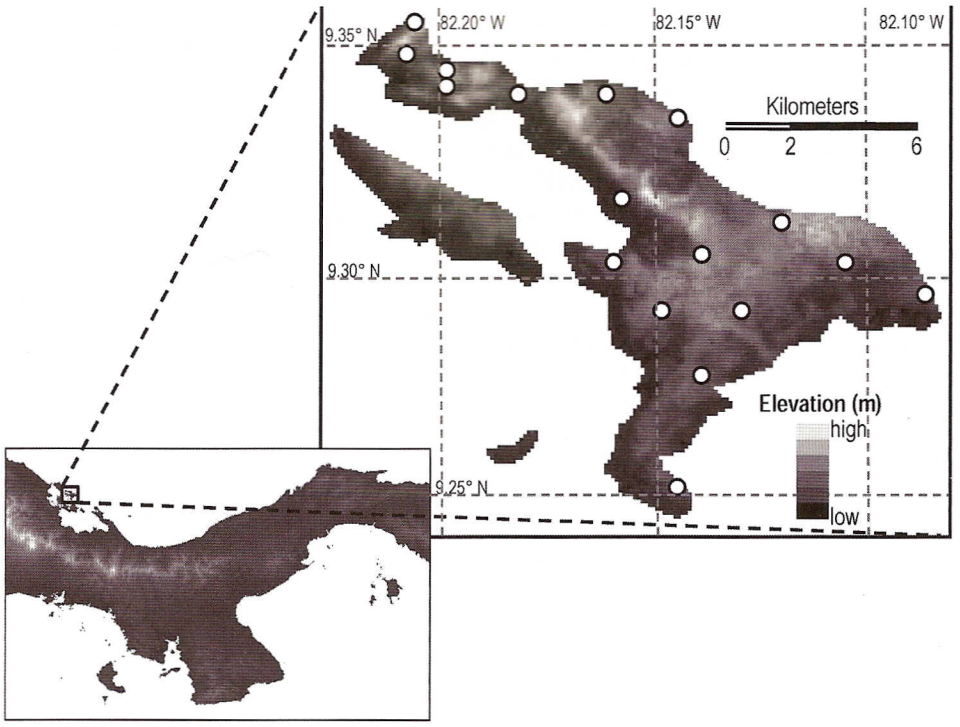

Fig. 1. Sampling locations for *Batrochochytrium dendrobatidis* in *Oophaga pumilio* frogs across Bastimentos Island, Panama.

Methods.—We captured adults from the leaf-litter at 17 transect points across Bastimentos Island (Fig. 1) during July 2007. Sampling locations were chosen to ensure all areas of the island (approximately every 2 km) were assessed for the presence of *Bd*. The dorsum, venter, and feet of five adults from each location were swabbed with a sterile cotton swab for a total of 85 individuals. Swabs were stored in dry microcentrifuge tubes and upon returning to the lab were refrigerated at 4°C until extraction. Individuals were released at the site of capture.

Extraction of *Bd* DNA was accomplished using the methodology of Hyatt et al. (2007). Taqman diagnostic quantitative PCR (Boyle et al. 2004) was used to detect presence of *Bd* DNA. Quantitative Taqman PCR assays were performed in triplicate using an Applied Biosystems Prism 7700 Sequence Detection System following the protocol of Boyle et al. (2004). VIC_{TM} Exogenous Internal Positive Control reagents were used for the detection of PCR inhibitors (Applied Biosystems following Hyatt et al. 2007). A sample was only considered positive for *Bd* if all three replicates indicated a presence of the fungus. Prevalence rates were calculated by dividing the number of infected individuals by the total number of sampled individuals, and 95% confidence intervals were calculated based on a binomial distribution (Stata Intercooled v. 10.0).

Results and Discussion.—None of the 85 individuals sampled tested positive for the presence of *Bd* in any of the three replicates (95% confidence interval = 0–4.2%). Inhibitors did not appear to be present in any of the samples. Our results suggest that there is either a very low level of *Bd* prevalence or that *Bd* is absent from the Island of Bastimentos. We have two hypotheses as to why *Bd* was not detected in our study. First, *Bd* may not yet have reached Bastimentos Island. The geographic isolation of Bastimentos *O. pumilio* populations from mainland populations may have impeded the spread of the disease. The island is, however, heavily traveled by tourists, implying that populations on these islands may be more connected to mainland populations than expected by geography alone.

Alternatively, the apparent absence of *Bd* on Bastimentos could be due to unsuitable environmental conditions. *Bd* is limited by temperatures outside the range of 4–25°C under laboratory conditions (Piotrowski et al. 2004), and the distribution and severity of infections appear to be correlated with rainfall and temperature patterns in wild populations (Kriger et al. 2007). However, *Bd* has been detected in at least one population of Panamanian frogs that

TABLE 1. Climate data for Bastimentos Island, Panama (Hijmans et al. 2005).

Climate Variable	Value
Annual Mean Temperature	25.8°C
Maximum Temperature of the Warmest Month (April)	30.0°C
Minimum Temperature of the Coldest Month (February)	21.0°C
Annual Temperature Range	9.0°C
Annual Precipitation	3109 mm
Precipitation of the Wettest Month (July)	398 mm
Precipitation of the Driest Month (March)	146 mm

is, on average, exposed to slightly hotter conditions than are *O. pumilio* populations on Bastimentos Island (C. L. Richards, pers. comm.). In addition, the average temperature and precipitation on Bastimentos Island (Hijmans et al. 2005; Table 1) is within the range of values for a number of infected sites in Central America (Ron 2005). It is therefore predicted that if *Bd* was introduced to these populations that it would be able to survive.

Identification of areas where *Bd* is absent is crucial for determining the physiological limits of the pathogen and for establishing areas of priority for conservation. Further research on the distribution of *Bd* will provide necessary information for predicting the patterns of spread, potentially assisting managers in preventing outbreaks of the disease.

Acknowledgments.—We thank the staff at the Smithsonian Tropical Research Institute (STRI) for their help with logistics and obtaining permits as well as Deanna Olson and Eli Greenbaum for providing useful comments that helped improve the manuscript. In conducting this research, we have complied with all applicable institutional Animal Care guidelines and obtained all required permits. This research was funded in part by a STRI Short Term Fellowship to CLR.

LITERATURE CITED

BERGER, L., R. SPEARE, P. DASZAK, D. E. GREEN, A. A. CUNNINGHAM, C. L. GOGGIN, R. SLOCOMBE, M. A. RAGAN, A. D. HYATT, K. R. MCDONALD, H. B. HINES, K. R. LIPS, G. MARANTELLI, AND H. PARKES. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. 95:9031–9036.

BOYLE, D. G., D. B. BOYLE, V. OLSEN, J. A. T. MORGAN, AND A. D. HYATT. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Org. 60:141–148.

GARNER, T. W. J., M. W. PERKINS, P. GOVINDARAJULU, D. SEGLIE, S. WALKER, A. A. CUNNINGHAM, AND M. C. FISHER. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biol. Lett. 2:455–459.

HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005 Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25:1965–1978.

HYATT, A. D., D. G. BOYLE, V. OLSEN, D. B. BOYLE, L. BERGER, D. OBENDORF, A. DALTON, K. KRIGER, M. HERO, H. HINES, R. PHILLOTT, R. CAMPBELL, G. MARANTELLI, F. GLEASON, AND A. COLLING. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 73:175–192.

KRIGER, K. M., F. PEREOGLOU, AND J. M. HERO. 2007. Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. Conserv. Biol. 21:1280–1290.

LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, C. CAREY, L. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Natl. Acad. Sci. 103:3165–3170.

PIOTROWSKI, J. S., S. L. ANNIS, AND J. E. LONGCORE. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15.

RON, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica 37:209–221.

SKERRATT, L. F., L. BERGER, R. SPEARE, S. CASHINS, K. R. MCDONALD, A. D. PHILLOTT, H. B. HINES, AND N. KENYON. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

WALLS, J. G. 1994. Jewels of the Rainforest – Poison Frogs of the Family Dendrobatidae. J.F.H. Publications, Neptune City, New Jersey.

WELDON, C., L. H. DU PREEZ, A. D. HYATT, R. MULLER, AND R. SPEARE. 2004. Origin of the amphibian chytrid fungus. Emerg. Infect. Dis. 10:2100–2105.

WOODHAMS, D. C., L. A. ROLLINS-SMITH, R. A. ALFORD, M. A. SIMON, AND R. N. HARRIS. 2007a. Innate immune defenses of amphibian skin: antimicrobial peptides and more. Anim. Conserv. 10:425–428.

———, V. T. VREDENBURG, M. A. SIMON, D. BILLHEIMER, B. SHAKHTOUR, Y. SHYR, C. J. BRIGGS, L. A. ROLLINS-SMITH, AND R. N. HARRIS. 2007b. Symbiotic bacteria contribute to innate immune defenses of the threatened mountain yellow-legged frog, *Rana muscosa*. Biol. Conserv. 138:390–398.

Herpetological Review, 2008, 39(2), 202–204.
© 2008 by Society for the Study of Amphibians and Reptiles

Results of Amphibian Chytrid (*Batrachochytrium dendrobatidis*) Sampling in Denali National Park, Alaska, USA

TARA CHESTNUT*
Washington State Department of Transportation, Olympic Region Environmental and Hydraulic Services, Tumwater, Washington 98504, USA

JAMES E. JOHNSON
and
R. STEVEN WAGNER
Department of Biological Sciences, Central Washington University Ellensburg, Washington 98925 USA

**Corresponding author: tarachestnut@gmail.com*

The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been detected in amphibian populations along the northwest coast of North America from Vancouver Island, British Columbia (Adams et al. 2008) north to the Kenai Peninsula (Reeves and Green 2006). However, *Bd* has not been detected in interior Alaska in the Innoko or Tetlin National Wildlife Refuges (Reeves 2008). The Wood Frog (*Rana sylvatica*) is the only amphibian species that occurs in interior Alaska (Wright and Wright 1995) and is susceptible to *Bd* infection (Reeves and Green 2006; Ouellet et al. 2005). In 2006, we sought to determine if *Bd* occurred in Wood Frogs in Denali National Park.

Methods.—Denali National Park (DNP) is located in central Alaska, approximately 183 km S of Fairbanks and 317 km N of Anchorage in Denali Borough (63.97°N, 149.13°W), and covers 2.4 million ha (Fig. 1). Three areas were surveyed for Wood Frogs within the park boundary: Wonder Lake vicinity roadside and backcountry, Teklanika River vicinity roadside, and along the roadside between the park entrance and the Savage River.

Known Wood Frog pond sites were surveyed in the Wonder Lake area (Hokit and Brown 2006), and seven additional ponds with no Wood Frog survey history were selected along the roadside in the Teklanika River vicinity and between the park entrance and the Savage River for their high traffic location. Surveys took place during a two-week period in August 2006 using standard techniques (Olson et al. 1997). Non-invasive techniques were used to