SHORT COMMUNICATIONS

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Survey for the Pathogenic Chytrid Fungus *Batrachochytrium dendrobatidis* in Southwestern North Carolina Salamander Populations

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ABSTRACT: *Batrachochytrium dendrobatidis* is a fungal pathogen responsible for a potentially fatal disease of amphibians. We conducted a survey for *B. dendrobatidis* in the Appalachian Mountains of southwestern North Carolina, USA, from 10 June to 23 July 23 2009. Ventral skin swabs were collected from plethodontid salamanders ($n=278$) and real-time PCR was performed to test for the presence of *B. dendrobatidis*. We found no evidence of *B. dendrobatidis*, suggesting that *B. dendrobatidis* is absent or present in such low levels that it was undetected. If *B. dendrobatidis* was present at the time of our sampling, this survey supports evidence of low prevalence of *B. dendrobatidis* in North American headwater stream salamander populations.

Key words: *Batrachochytrium dendrobatidis*, Coweeta, North Carolina, salamander.

Chytridiomycosis is an emerging infectious disease contributing to the global decline of amphibian populations (Berger et al., 1998). Amphibians become infected when zoospores produced by the fungus *Batrachochytrium dendrobatidis* disperse in water and infect keratinized epidermis of individuals, although mortality typically does not occur until metamorphosis (Berger et al., 2005). The degree to which species display signs of chytridiomycosis is variable, with some species remaining subclinical after infection (Vazquez et al., 2009).

Although *B. dendrobatidis* is widely distributed in the southeastern USA, chytridiomycosis has not been directly linked with amphibian population declines in this region. The fungus has been correlated with population declines in the western USA, Central America, Europe, and Australia (Berger et al., 1998; Bosch et al., 2001; Muths et al., 2003; Rothermel et al., 2008). Although salamanders do not seem to be affected as severely by chytridiomycosis as many anuran species, mortality and sublethal effects (e.g., weight loss) have been observed in several salamander species (Chinnadurai et al., 2009; Vazquez et al., 2009). Therefore, it seems that at least some salamander populations are potentially vulnerable to *B. dendrobatidis*. The southern Appalachian Mountain region is an area of high salamander diversity and endemism (Duellman and Sweet, 1999). Unfortunately, knowledge of the prevalence of *B. dendrobatidis* in salamander populations in this region is limited. Our objective was to assess the prevalence of *B. dendrobatidis* in several plethodontid species associated with small, headwater streams in southwestern North Carolina.

Surveys were conducted at the Coweeta Hydrologic Laboratory (hereafter Coweeta; 35°03′N, 83°25′W), a long-term ecological research site located in the Nantahala Mountains, Macon County, North Carolina (Swank and Crossley, 1988). Coweeta is a 2,185-ha predominantly forested site at an elevation of 675–1,592 m that contains a dendritic network of first- and second-order streams (Swank and Crossley, 1988). The climate is predicted to be suitable for *B. dendrobatidis* (Ron, 2005).

Larval and adult salamanders were captured in six permanent first- or second-order streams from 10 June to 23 July 2009 (Fig. 1). Larvae were sampled using

10 plastic mesh litter bags (70×70 cm) filled with leaf litter collected from stream banks and placed individually in stream pools every 10 m (Waldron et al., 2003). Litter bags were secured in place by using large rocks and checked approximately biweekly. Adult salamander surveys were conducted at least 1 hr after dusk. During these surveys two researchers slowly searched for surface-active adults along a 10-m stretch of the stream, including approximately 1 m of riparian areas adjacent to streams. Adults were captured by hand or "fished" from stream bank refugia (Camp and Lovell, 1989). A different 10-m stretch was sampled on each visit so it was unlikely that the same individual was sampled more than once because of the small home range size of many salamander species (Peterman et al., 2008). Individuals were identified to species and swabbed to test for the presence of *B. dendrobatidis* before being returned to their site of capture.

Samples to test for *B. dendrobatidis* were obtained from *Desmognathus monticola* (10 adults), *Desmognathus ocoee* (53 adults), *Desmognathus quadramaculatus* (132 adults, five larvae), *Eurycea wilderae* (73 larvae), *Gyrinophilus porphyriticus* (four larvae), and *Plethodon shermani* (one adult). All of these species except for *P. shermani* have an aquatic larval stage and adults that use stream habitats to varying degrees and may therefore encounter zoospores of *B. dendrobatidis*. Individuals were transported to the laboratory in 0.95-l Ziploc® bags containing approximately 500 ml of stream water maintained at ~25 C. Adults were kept individually in bags, but up to 15 larvae of the same species were occasionally kept in the same bag as part of another study. Individuals were then transferred to a new Ziploc® bag to collect *B. dendrobatidis* samples. Keeping individuals cool and bagged reduced their activity and made them easier to handle. Samples were collected by rubbing the complete ventral surface of an individual with fine-tipped rayon swabs (Dryswabs™ MW113, Medical Wire & Equipment Co., Corsham, Wiltshire, England) at least 20 times. Swab samples were air-dried approximately 5 min, placed individually into 2-ml plastic vials (VWR, West Chester, Pennsylvania, USA), and frozen (approximately −20 C). Samples were shipped overnight on ice to the Amphibian Disease Laboratory at the San Diego Zoo's Institute for Conservation Research (San Diego, California, USA). DNA template for real-time Taqman PCR was prepared from swabs using PrepMan® Ultra (Applied Biosystems, Foster City, California, USA). PCR primers, probe, exogenous internal positive controls, cycling conditions, chemistry, and quantities of sample DNA were as described previously (Hyatt et al., 2007). Reactions were run in triplicate on a 7900 Real-Time PCR System (Applied Biosystems). Positive controls and standard curves were constructed using 10-fold serial dilutions of cultured *B. dendrobatidis* zoospores.

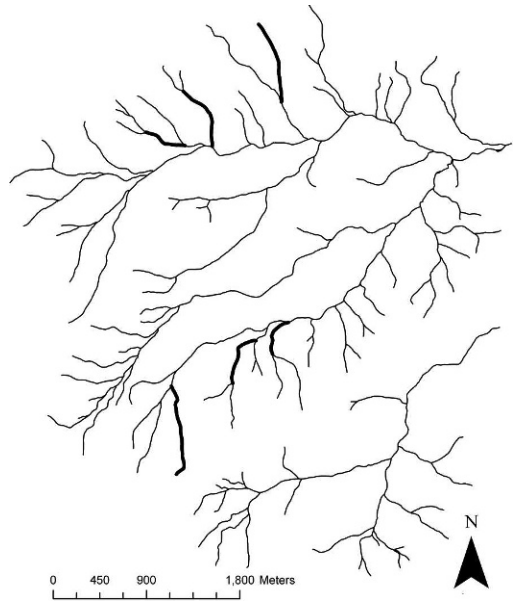

FIGURE 1. Location of sampling sites for *Batrachochytrium dendrobatidis* surveys. The first- and second-order headwater streams surveyed were located at Coweeta Hydrologic Laboratory in Macon, County, North Carolina, USA.

We did not detect *B. dendrobatidis* in swabs from any species. One possible

explanation of this is that the species sampled are resistant to infection. This is unlikely because *B. dendrobatidis* has been observed in congeneric species, and several plethodontid species have been experimentally infected (Chinnadurai et al., 2009; Vazquez et al., 2009). In addition, *B. dendrobatidis* has been observed in a broad range of amphibian hosts (>200 species; Aanensen, 2010), and it is likely that many salamander species are potential hosts as well.

A second possibility is that *B. dendrobatidis* was present but not detected. Reports of *B. dendrobatidis* from watersheds in surrounding areas indicate that the watersheds we sampled are suitable habitat for *B. dendrobatidis* and suggest that it may have been present (Chatfield et al., 2009; Chinnadurai et al., 2009). We sampled a large number of larval salamanders, which may have limited our ability to detect *B. dendrobatidis*. Larval salamanders have relatively little keratinized epidermis, which limits the colonizable area for *B. dendrobatidis*. In addition, several species of salamanders contain cutaneous bacteria that produce antifungal metabolites that inhibit fungal infections, including *B. dendrobatidis* (Harris et al., 2006). We do not know whether the salamander species we sampled contain such bacteria; if so, this may have reduced the amount of *B. dendrobatidis* on individuals and limited our ability to detect it. There is also evidence that *B. dendrobatidis* can be present in populations of headwater stream salamanders at low levels that can be difficult to detect during surveys (Hossack et al., 2010). Given that the watersheds we sampled seem to be suitable habitat for *B. dendrobatidis*, the potential sampling issues with larval salamanders, and the apparently general low prevalence of *B. dendrobatidis* in salamander populations, it is possible that *B. dendrobatidis* was present but in such low levels that we failed to detect it.

A third possibility is that *B. dendrobatidis* was not present in these watersheds at the time of sampling. Assuming a low prevalence of 1% might have existed, there was a 94% probability that we would have detected at least one positive individual by sampling 278 individuals as calculated using Win Episcope 2.0 (CLIVE, Edinburgh, UK). Surveys from other North American headwater stream amphibian populations (including 18 salamander species) indicate a prevalence of about 3% (Hossack et al., 2010). Assuming a similar prevalence in the watersheds we sampled, the probability of detecting at least one positive would be close to 100%. It is therefore possible that *B. dendrobatidis* is absent from the watersheds we sampled. However, given that *B. dendrobatidis* has been found in nearby watersheds and the potential sampling issues discussed above, we are not confident in ruling out the possibility that it is present. Rather, we believe that if *B. dendrobatidis* is present, it is at such a low prevalence that we were unable to detect it.

Our results provide support for the growing body of evidence that if *B. dendrobatidis* is present in headwater stream salamander populations, it is present in such low levels that it can be difficult to detect (Hossack et al., 2010). Our results also may indicate that *B. dendrobatidis* is absent from the watersheds we sampled, but further sampling is needed to verify this possibility. The southern Appalachian Mountains represent an area of high salamander diversity and endemism and further research is needed to determine the potential effects of *B. dendrobatidis* on salamander populations in this area.

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