The Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis*, in Fully Aquatic Salamanders from Southeastern North America

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Abstract

Little is known about the impact that the pathogenic amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has on fully aquatic salamander species of the eastern United States. As a first step in determining the impacts of *Bd* on these species, we aimed to determine the prevalence of *Bd* in wild populations of fully aquatic salamanders in the genera *Amphiuma*, *Necturus*, *Pseudobranchus*, and *Siren*. We sampled a total of 98 salamanders, representing nine species from sites in Florida, Mississippi, and Louisiana. Overall, infection prevalence was found to be 0.34, with significant differences among genera but no clear geographic pattern. We also found evidence for seasonal variation, but additional sampling throughout the year is needed to clarify this pattern. The high rate of infection discovered in this study is consistent with studies of other amphibians from the southeastern United States. Coupled with previously published data on life histories and population densities, the results presented here suggest that fully aquatic salamanders may be serving as important vectors of *Bd* and the interaction between these species and *Bd* warrants additional research.

Introduction

The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), has been associated with amphibian declines and extinctions worldwide [1–5]. The severity of impact varies greatly across species, with some species undergoing greater declines (e.g., frogs in the genus *Atelopus* [6]) than others (e.g., proposed carrier species such as *Lithobates catesbeianus* [7] and *L. pipiens* [8]). The impact of *Bd* on many groups, especially semi-aquatic frogs, is well documented; however, the impact of *Bd* on aquatic salamanders is not well understood.

Determining the prevalence of *Bd* in wild populations is a necessary first step in determining the impact that *Bd* has on those populations. Currently, little is known about the prevalence of *Bd* in fully aquatic salamanders. *Bd* has been detected on three species of wild-caught, fully-aquatic salamanders: *Cryptobranchus alleganiensis* [9–13], a species which has recently been listed under Appendix III of the Convention on International Trade of Endangered Species (CITES) and a subspecies of which (*C. a. bishop*) has been listed under the U. S. Endangered Species Act; *Amphiuma japonicus* [14], which is currently listed under Appendix I of CITES; and a single individual *Siren intermedia* from Illinois [15]. In captivity, *Bd* is known from an additional three species: *Amphiuma tridactylum*, *Necturus marmoratus* and *Siren lacertina* [16].

Salamanders in the genera *Amphiuma* (3 species), *Pseudobranchus* (2 species), *Siren* (2 species) and *Necturus* (5 species) are fully aquatic and have distributions that are restricted to eastern North America, with the greatest diversity being found along the coastal plain of the southeastern United States. Despite their restricted distributions and their evolutionary and ecological uniqueness, little attention has been given to these groups with respect to *Bd*. In this study, we report the results of a survey identifying the presence and prevalence of *Bd* in salamanders representing all four of these aquatic genera from locations in Florida, Mississippi, and Louisiana.

Methods

Ethics Statement

A permit was obtained through the Louisiana Department of Wildlife and Fisheries (permit number LNHP-11-025) for collections made in that state. No permit was obtained for sampling performed in Mississippi and Florida, as those states do not require permits for sampling amphibian species not listed as threatened or endangered. Animal use protocols were approved by Tulane University’s Institutional Animal Care and Use Committee (protocol number 0411).

Field surveys for salamanders were conducted at locations in southeastern Louisiana (East Baton Rouge, St. John the Baptist, Tangipahoa and St. Tammany parishes), southern Mississippi (Forrest County) and the panhandle and northern peninsula of Florida (Escambia, Okaloosa, Washington, Liberty, Levy and Putnam counties) (Figure 1). Animals were captured by dipnetting and trapping (baited and unbaited minnow traps placed in suitable...
prior to use. In addition, 0.7 mL of bovine serum albumin (BSA) was added to each reaction well, as this has been shown to aid in overcoming problems with inhibition [23]. All samples were run in triplicate and scored as positive if at least one replicate tested positive for Bd. To confirm that reactions were not inhibited, an internal positive control (VICTM dye, Applied Biosystems, Inc.) was added to one replicate of each sample. Upper and lower limits of the 95% confidence interval (CI95) for prevalence were calculated according to Newcombe [24].

### Results

The prevalence of Bd among all salamanders surveyed in this study was 0.34 (CI95 = 0.25 – 0.43, Table 1), although prevalence differed among genera (Fisher’s Exact Test: P = 0.009, Figure 2). *Necturus* (N = 12, prevalence = 0.42, CI95 = 0.19 – 0.68) and *Amphiuma* (N = 30, prevalence = 0.516/L, following extraction and open circles indicate those sites where *Bd* was not detected. Background shading indicates elevation (lighter = higher elevation).

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Figure 1. Map of southeastern United States showing collection localities. Filled circles indicate those sites where *Bd* was detected and open circles indicate those sites where *Bd* was not detected. Background shading indicates elevation (lighter = higher elevation).

### Table 1. Results of survey for *Batrachochytrium dendrobatidis* in fully aquatic salamanders from Florida, Louisiana, and Mississippi.

| Species                  | Site | Lat/Long | State | N | # Positive | Prev. |
|--------------------------|------|----------|-------|---|------------|-------|
| *Amphiuma means*         | E    | 31.154/–89.245 | MS    | 4 | 1          | 0.25  |
| *Amphiuma*               | F    | 31.113/–89.142  | MS    | 14| 1          | 0.07  |
| *Necturus alabamensis*   | J    | 30.248/–85.005  | FL    | 3 | 0          | 0     |
| *N. alabamensis*         | K    | 30.259/–84.972  | FL    | 2 | 0          | 0     |
| *N. bayeri*              | D    | 30.539/–89.876  | LA    | 2 | 1          | 0.5   |
| Total *Necturus*         |      |           |       | 55| 23         | 0.4   |
| *Pseudobranchus axanthus*| O    | 29.542/–81.837  | FL    | 13| 1          | 0.08  |
| *P. striatus*            | I    | 30.789/–85.756  | FL    | 1 | 0          | 0     |
| Total *Pseudobranchus*   |      |           |       | 14| 1          | 0.07  |
| *Siren intermedia*       | B    | 30.397/–90.429  | LA    | 1 | 0          | 0     |
| *S. intermedia*          | C    | 30.108/–90.435  | LA    | 1 | 1          | 1     |
| *S. intermedia*          | E    | 31.154/–89.245  | MS    | 1 | 1          | 1     |
| *S. lacertina*           | F    | 31.113/–89.142  | MS    | 2 | 0          | 0     |
| *S. lacertina*           | O    | 29.542/–81.837  | FL    | 3 | 0          | 0     |
| *S. lacertina*           | L    | 29.516/–82.876  | FL    | 3 | 2          | 0.67  |
| *Siren sp.*              | G    | 30.516/–87.322  | FL    | 1 | 1          | 1     |
| Total *Siren*            |      |           |       | 12| 5          | 0.42  |
| Total Salamanders        |      |           |       | 98| 33         | 0.34  |

*Sites correspond to those given in Figure 1.

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Discussion

Prevalence of Bd infection in the fully aquatic salamander species was high (0.34 with a CI95 of 0.25–0.43) during the period sampled in this study. The peak infection period observed in this study (March–May) corresponds well to the peak infection period observed in other (semi-aquatic) species in this region [17–21]. The sharp decline in prevalence in June may indicate the start of a season of low prevalence that is also seen in semi-aquatic species in this region [21]. It is important to note, however, that sampling in July and August was limited to 13 individuals collected from one site in Mississippi. Additional sampling is needed to fully address the issue of seasonality.

Alternatively, the greater thermal stability of aquatic environments suggests that fully aquatic amphibian species might be year-round hosts of Bd, serving as a reservoir for the pathogen that could infect semi-aquatic species during their breeding season. The preferred environmental temperatures of two species of fully aquatic salamanders, Cryptobranchus alleganiensis and Necturus maculosus (11.6–21.7°C and 9.1–20.2°C, respectively, [25] overlap with the lower end of the optimal growth range of Bd (17–25°C [26–29]). The preferred temperature of a third species, Amphiuma tridactylum, is marginally higher (26.3°C [30]), although still within the range where Bd can survive [28]. Species of Amphiuma also rely heavily on crayfish burrows as retreat sites [30]. These microhabitats provide suitable environments for Bd growth (18–20°C [30]) and may, in conjunction with aestivating Amphiuma, be acting as...
reservoirs for Bd during warm summer months in subtropical climates and, thus, may contribute to the high prevalence of Bd (0.40) found in Amphiuma in this study. Lastly, Siren and Pseudobranchus are able to survive in seasonal wetlands or periods of drought by burrowing into the substrate and forming a desiccation-resistant cocoon [31–33]. As with Amphiuma, aestivating Siren and Pseudobranchus may be acting as Bd reservoirs. Additional research is needed to elucidate the role that these complex adaptations play in Bd-host interactions.

We did not detect any geographic patterns with respect to the prevalence or prevalence of Bd infection. Positive individuals were detected in all three of the states sampled and prevalence was not significantly different across states. Importantly, all of our sites were of similar latitude (29.5–31.2° N) and likely experienced similar climates. Other studies conducted in the southeastern United States support the widespread distribution of Bd in this region and have found similarly high infection prevalences in other amphibian species during certain times of the year. For example, Gaertner et al. [18] found an infection prevalence of 0.83% in cricket frogs (Acris crepitans) sampled in May from a site in central Texas. In Virginia, Pullen et al. [19] found a peak prevalence of 0.45% across 13 semi-aquatic frog and salamander species. Sampling across seasons, Rothermel et al. [17] found a prevalence of 0.18% in 12 species across four sites in Georgia, North Carolina, and South Carolina.

The high prevalence of Bd found in this study is significant and suggests that additional studies are needed to understand the impact of Bd on these taxa. The absence of Bd-related die-offs of amphibians in eastern North America suggests that Bd is acting in an endemic fashion in the region. Currently, little is known about the effects of Bd on amphibian populations in the absence of mass die-offs; however, previous studies have demonstrated a negative impact of Bd on semi-aquatic frog species following historic mass die-offs [34] and altered anti-predator defense strategies in Bd-infected versus uninfected tadpoles (e.g., [35]). The Ozark Hellbender (Cryptobranchus alleganiensis bispinis), another fully aquatic salamander from the eastern United States, has recently been listed as federally endangered and also has a high reported prevalence of Bd in the wild (0.33) [13]. While it is difficult to ascertain the role that Bd has played in the decline of that species, it seems likely that many stressors acting in concert may be reducing survival and reproduction (e.g., [12], [36]). Given the presence of Bd infection in fully aquatic salamanders, the threat that Bd poses will ultimately depend upon the extent to which these groups exhibit symptoms of chytridiomycosis, the disease caused by Bd, or other subclinical effects. Some terrestrial and semi-aquatic salamander species are known to harbor antifungal bacteria and compounds on their skin [37–39]. While many fully aquatic salamander species are commonly known to have abundant mucus secretions, research is needed to identify whether these secretions contain antifungal compounds active against Bd.

Lastly, the extraordinarily high densities reached by many species of fully aquatic salamanders in the wild suggest that these species may play a disproportionately large role in Bd-amphibian interactions. For example, populations of Siren lacertina and Amphiuma means in northern Florida have been found to reach densities of 1.3 and 0.29 salamanders/m², respectively [40]; and populations of Siren intermedia in Texas and Missouri have densities of 0.33–1.1 and 1.35–2.17 salamanders/m², respectively [41–43]. Furthermore, many of the species examined in this study are large, making them significant contributors to biomass in southeastern aquatic ecosystems. For example, estimates of the standing crop biomass of S. lacertina is 253 g/m², A. means is 44 g/m², and S. intermedia is 9.66–72.2 g/m² [40–43]. The high densities at which these salamanders occur in nature suggests that fully aquatic salamanders of the eastern United States are ecologically important. Therefore, understanding potential threats to these species is important in the conservation and management of aquatic ecosystems in this region. Additionally, these species may be harboring large amounts of Bd, thus contributing disproportionately as vectors to Bd-host interactions involving other (semi-aquatic) amphibian groups.

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Author Contributions

Conceived and designed the experiments: MWHC. Performed the experiments: MWHC PM. Analyzed the data: MWHC CLR-Z. Contributed reagents/materials/analysis tools: CLR-Z. Wrote the paper: MWHC.

References

1. Berger L, Speare R, Daszak P, Green DE, Cunningham AA, et al. (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc Natl Acad Sci USA 95: 9031–9036.
2. Daszak P, Cunningham AA, Hyatt AD (2003) Infectious disease and amphibian population declines. Divers Distrib 9: 141–150.
3. Mendelson JR, Lips KR, Gaßlhofer RW, Rabb GB, Collins JP, et al. (2006) Biodiversity: Confronting amphibian declines and extinctions. Science 313: 48.
4. Lips KR, Brum F, Briones R, Reese JD, Alford RA, et al. (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc Natl Acad Sci USA 103: 3161–3170.
5. Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, et al. (2007) Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4: 125–134.
6. La Marca E, Lips KR, Lotters S, Puscheckend K, Bä dzie R, et al. (2005) Catastrophic population declines and extinctions in neotropical harlequin frogs (Atelopus). Science 310: 931–934.
7. Daszak P, Sristy A, Cunningham AA, Longcore JE, Brown CC, et al. (2004) Experimental evidence that the bullfrog (Rana catesbeiana) is a potential carrier of chytridiomycosis, an emerging fungal disease of amphibians. Herp J 14: 201–207.
8. Woodhams DC, Hyatt AD, Boyle DG, Rollins-Smith LA (2008a) The Northern Leopard Frog Rana pipiens is a widespread reservoir species harboring Batrachochytrium dendrobatidis in North America. Herp Rev 39: 66–68.
9. Briggler JT, Efting J, Wanner M, Schuette C, Duncan M, et al. (2007) Cryptobranchus alleganiensis (hellbender). Chytrid fungus. Herpetological Review 38:174.
10. Briggler JT, Larson KA, Irvin KJ (2008) Presence of the amphibian chytrid fungus Batrachochytrium dendrobatidis on hellbenders (Cryptobranchus alleganiensis) in the Ozark Highlands. Herp Rev 39: 443–444.
11. Bodinof CM, Briggler JT, Duncan MC, Beringer J, Millerhouse JJ (2011) Historic occurrence of the amphibian chytrid fungus Batrachochytrium dendrobatidis in hellbender Cryptobranchus alleganiensis populations from Missouri. Dis Aquat Org 96: 1–7.
12. Burgmeier ND, Unger SD, Meyer JL, Sutton TM, Williams RN (2011) Health and habitat quality assessment for the eastern hellbender Cryptobranchus alleganiensis alleganiensis in Indiana, USA. J Wild Dis 47: 836–840.
13. Gonyon JR, Yabsley MJ, Jensen JR (2011) A preliminary survey of Batrachochytrium dendrobatidis exposure in hellbenders from a stream in Georgia, USA. Herp Rev 42: 58–59.
14. Goke K, Yokoza H, Uy Y, Kusuki T, Suzuki K, et al. (2009) Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Mol Ecol 18: 4757–4774.
15. Talley BL, Lips KR, SR Ballard (2011) Batrachochytrium dendrobatidis in Siren intermedia in Illinois, USA. Herp Rev 42: 216–217.
16. Speare R, Berger L (2000) Global distribution of chytridiomycosis in amphibians. Available at: http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglf.htm. 11 November 2000.
