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

Matthew W. H. Chatfield[1]*, Paul Moler[2], Corinne L. Richards-Zawacki[1]

[1] Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, Louisiana, United States of America, [2] Wildlife Research Laboratory, Florida Fish and Wildlife Conservation Commission, Gainesville, Florida, United States of America

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.

Citation: Chatfield MWH, Moler P, Richards-Zawacki CL (2012) The Amphibian Chytrid Fungus, *Batrachochytrium dendrobatidis*, in Fully Aquatic Salamanders from Southeastern North America. PLoS ONE 7(9): e44821. doi:10.1371/journal.pone.0044821

Editor: Joy Sturtevant, Louisiana State University, United States of America

Received February 27, 2012; **Accepted** August 14, 2012; **Published** September 11, 2012

Funding: This study was funded by Tulane University through start-up funds given to C.L.R.-Z. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mattchat@tulane.edu

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; *Andrias 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 maculosus* 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

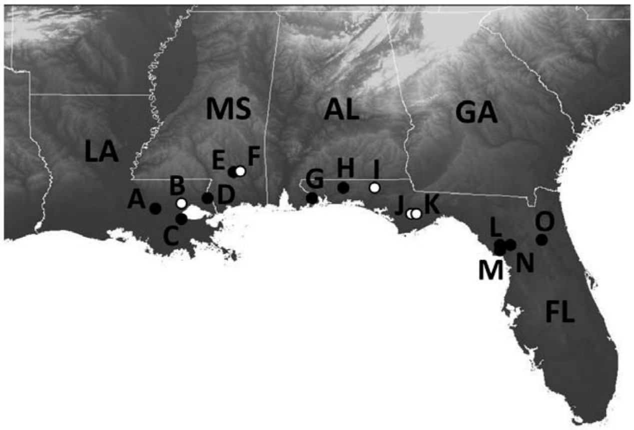

Figure 1. Map of southeastern United States showing collection localities. Filled circles indicate those sites where *Bd* was detected and open circles indicate those sites where *Bd* was not detected. Background shading indicates elevation (lighter = higher elevation).
doi:10.1371/journal.pone.0044821.g001

habitat overnight). As seasonality has been shown to influence *Bd* prevalence in semi-aquatic species, sampling efforts were focused on the peak prevalence period of March-June [17–21].

In order to test for *Bd* infection, salamanders were swabbed by gently rubbing a cotton-tipped swab (MWE113, Advantage Bundling SP, LLC, Durham, NC) 5 times over the dorsum, 5 times on each side, 5 times on the venter, and 5 times on the bottom of each foot. Clean nitrile gloves were used when handling animals, and changed between animals. DNA was extracted from the swabs using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, CA) in a final elution volume of 200 µL, following the protocol for animal tissues.

Quantitative (real-time) PCR assays were used to detect the presence of *Bd* DNA, following Boyle et al. [22]. Preliminary tests showed that PCR inhibition was often present; therefore, all extracted samples were diluted 1:10 with doubly deionized water prior to use. In addition, 0.7 µL 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 ($\mathrm{VIC}_{\mathrm{TM}}$ dye, Applied Biosystems, Inc.) was added to one replicate of each sample. Upper and lower limits of the 95% confidence interval (CI_{95}) for *Bd* prevalence were calculated according to Newcombe [24].

Results

The prevalence of *Bd* among all salamanders surveyed in this study was 0.34 ($\mathrm{CI}_{95} = 0.25–0.43$, Table 1), although prevalence differed among genera (Fisher's Exact Test: $P = 0.009$, Figure 2). *Siren* ($N = 12$, prevalence $= 0.42$, $\mathrm{CI}_{95} = 0.19–0.68$) and *Amphiuma* ($N = 55$, prevalence $= 0.40$, $\mathrm{CI}_{95} = 0.28–0.53$) had the highest *Bd* prevalence, followed by *Necturus* ($N = 17$, prevalence $= 0.24$, $\mathrm{CI}_{95} = 0.10–0.47$) and *Pseudobranchus* ($N = 14$, prevalence $= 0.07$, $\mathrm{CI}_{95} = 0.02–0.31$). Infections did not show any clear geographic pattern and *Bd* prevalence did not differ among states (Fisher's Exact Test: $P = 0.51$, Figure 3). Out of 98 individuals tested, 94 were captured from March through August (Figure 4). *Bd* prevalence differed across these six months with March ($N = 7$, prevalence $= 0.86$, $\mathrm{CI}_{95} = 0.49–0.97$), April ($N = 62$, prevalence $= 0.37$, $\mathrm{CI}_{95} = 0.26–0.50$) and May ($N = 3$, prevalence $= 0.67$, $\mathrm{CI}_{95} = 0.21–0.94$) having higher prevalence than June ($N = 9$, prevalence $= 0.11$, $\mathrm{CI}_{95} = 0.02–0.44$), July ($N = 5$, preva-

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
A. means	F	31.113/ −89.142	MS	14	1	0.07
A. means	N	29.326/ −82.772	FL	4	3	0.75
A. means	L	29.516/ −82.876	FL	8	8	1
A. means	M	29.286/ −82.845	FL	7	4	0.57
A. pholeter	G	30.516/ −87.322	FL	1	0	0
A. tridactylum	A	30.364/ −91.121	LA	11	2	0.18
Amphiuma sp.	I	30.789/ −85.756	FL	2	0	0
Amphiuma sp.	L	29.516/ −82.876	FL	4	4	1
Total *Amphiuma*				55	23	0.4
Necturus alabamensis	J	30.248/ −85.005	FL	3	0	0
N. alabamensis	K	30.259/ −84.972	FL	2	0	0
N. alabamensis	H	30.700/ −86.576	FL	10	3	0.3
N. beyeri	D	30.539/ −89.875	LA	2	1	0.5
Total *Necturus*				17	4	0.24
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. intermedia	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.
doi:10.1371/journal.pone.0044821.t001

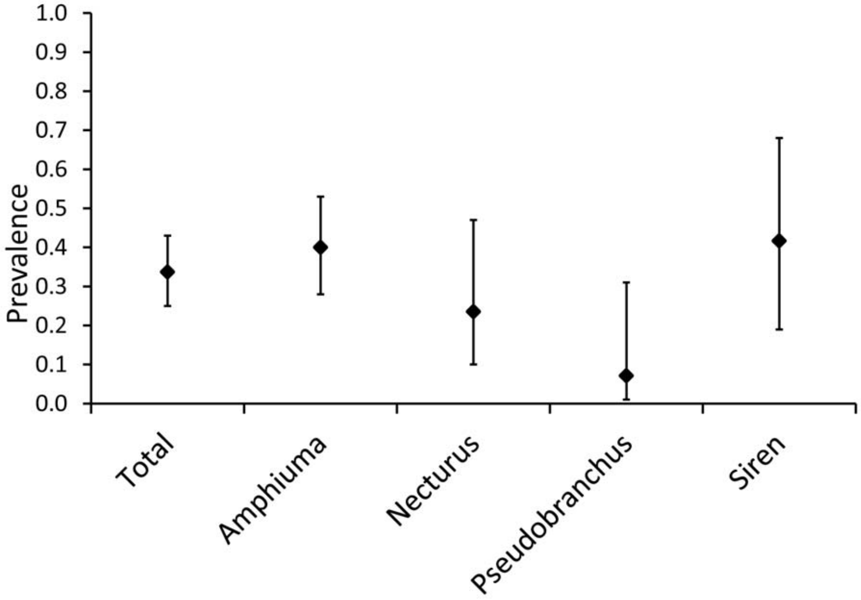

Figure 2. Prevalence of *Bd* infection in all genera combined (Total, N = 98), *Amphiuma* (N = 55), *Necturus* (N = 17), *Pseudobranchus* (N = 14), and *Siren* (N = 12). Bars indicate 95% confidence interval.
doi:10.1371/journal.pone.0044821.g002

lence = 0, CI_{95} = 0–0.43) or August (N = 8, prevalence = 0, CI_{95} = 0–0.32).

Discussion

Prevalence of *Bd* infection in the fully aquatic salamander species was high (0.34 with a CI_{95} 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

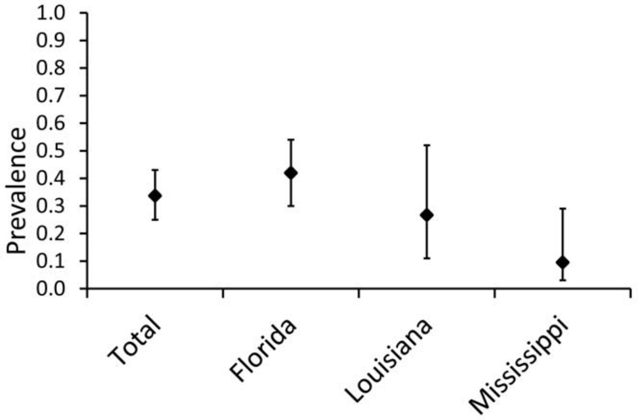

Figure 3. Prevalence of *Bd* infection in all sampling localities combined (Total, N = 98), Florida (N = 62), Louisiana (N = 15), and Mississippi (N = 21). Bars indicate 95% confidence interval.
doi:10.1371/journal.pone.0044821.g003

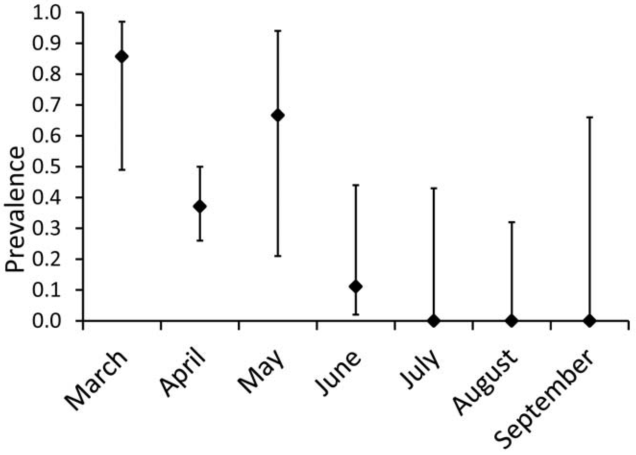

Figure 4. Prevalence of *Bd* infection for the six months in which sampling was heaviest: March (N = 7), April (N = 62), May (N = 3), June (N = 9), July (N = 5), and August (N = 8). Bars indicate 95% confidence interval.
doi:10.1371/journal.pone.0044821.g004

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 presence 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 bishopi*), 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 0.28 salamanders/m^2, respectively [40]; and populations of *Siren intermedia* in Texas and Missouri have densities of 0.33–1.1 and 1.35–2.17 salamanders/m^2, 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 233 g/m^2, *A. means* is 44 g/m^2, and *S. intermedia* is 9.66–72.2 g/m^2 [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.

Acknowledgments

We would like to thank J. Lee, J. Pojman, E. Rittmeyer, S. Slavens and C. Vance for help in the field and L. Brannelly for help in the lab.

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, Gagliardo RW, Rabb GB, Collins JP, et al. (2006) Biodiversity: Confronting amphibian declines and extinctions. Science 313: 48.
4. Lips KR, Brem F, Brenes R, Reeve 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: 3165–3170.
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, Lötters S, Puschendorf R, Ibáñez R, et al. (2005) Catastrophic population declines and extinctions in neotropical harlequin frogs (Bufonidae: *Atelopus*). Biotropica 37: 190–201.
7. Daszak P, Strieby A, Cunningham AA, Longcore JE, Brown CC, et al. (2004) Experimental evidence that the bullfrog (*Rana catesbieana*) 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, Ettling J, Wanner M, Schuette C, Duncan M, et al. (2007) *Cryptobranchus allaganiensis* (hellbender). Chytrid fungus. Herpetological Review 38:174.
10. Briggler JT, Larson KA, Irwin 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, Millspaugh JJ (2011) Historic occurance 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: 836–848.
13. Gonynor JL, Yabsley MJ, Jensen JB (2011) A preliminary survey of *Batrachochytrium dendrobatidis* exposure in hellbenders from a stream in Georgia, USA. Herp Rev 42: 58–59.
14. Goka K, Yokoyama J, Une Y, Kuroki 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/chyglob.htm. 11 November 2000.

17. Rothermel BB, Walls SC, Mitchell JC, Dodd Jr CK, Irwin LK, et al. (2008) Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. Dis Aquat Org 82: 3–18.
18. Gaertner JP, Gaston MA, Spontak D, Forstner MRJ, Dahn D (2009) Seasonal variation in the detection of *Batrachochytrium dendrobatidis* in a Texas population of Blanchard's Cricket Frog (*Acris crepitans blanchardi*). Herp Rev 40: 184–187.
19. Pullen KD, Best AM, Ware JL (2010) Amphibian pathogen *Batrachochytrium dendrobatidis* prevalence is correlated with season and not urbanization in central Virginia. Dis Aquat Org 91: 9–16.
20. Kinney VC, Heemeyer JL, Pessier AP, Lannoo ML (2011) Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality in *Lithobates areolatus*: affirmation of Vrendenberg's "10,000 Zoospore Rule". PLoS One 6: e16708.
21. Brannelly LA, Chatfield MWH, Richards-Zawacki CL (2012) Field and laboratory studies of *Hyla cinerea* susceptibility to *Batrachochytrium dendrobatids* infection. PLoS One 6:e38473.
22. Boyle DG, Boyle DB, Olsen V, Morgan JAT, Hyatt AD (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Org 60: 141–148.
23. Garland S, Baker A, Phillott AD, Skerratt LF (2010) BSA reduces inhibition in a TaqMan® assay for the detection of *Batrachochytrium dendrobatidis*. Dis Aquat Org 92: 113–116.
24. Newcombe RG (1998) Two-sided confidence intervals for the single proportion comparison of seven methods. Statistics in Medicine 17: 857–872.
25. Hutchison VH, Hill LG (1976) Thermal selection in the hellbender, *Cryptobranchus alleganiensis*, and the mudpuppy, *Necturus maculosus*. Herpetologica 32: 327–331.
26. Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. Nov., a Chytrid Pathogenic to Amphibians. Mycologia 91:219–227.
27. Johnson ML, Berger L, Philips L, Speare R (2003) Fungicidal effects of chemical disinfectants,UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. Dis Aquat Org 57:255–260.
28. Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15.
29. Woodhams D C, Alford RA, Briggs CJ, Johnson M, Rollins-Smith L (2008b) Life-history trade-offs influence disease in changing climates: strategies of an amphibian pathogen. Ecology 89:1627–1639.
30. Fontenot CL, Lutterschmidt WI (2011) Thermal selection and temperature preference of the aquatic salamander, *Amphiuma tridactylum*. Herp Cons Biol 6: 395–399.
31. Reno HW, Gehlbach FR, Turner RA (1972) Skin and aestivational cocoon of the aquatic amphibian, *Siren intermedia* Le Conte. Copeia 4: 625–631.
32. Gehlbach FR, Gordon R, Jordan JB (1973) Aestivation of the salamander, *Siren intermedia*.
33. Etheridge K (1990) The energetics of estivating sirenid salamanders (*Siren lacertina* and *Pseudobranchus striatus*). Herpetologica 46: 407–414.
34. Murray KA, Skerratt LF, Speare R, McCallum H (2009) Impact and dynamics of disease in species threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*. Cons Biol 23: 1242–1252.
35. Parris MJ, Reese E, Storfer A (2006) Antipredator behavior of chytridiomycosis-infected northern leopard frog (*Rana pipiens*) tadpoles. Can J Zool 84: 58–65.
36. Foster RL, McMillan AM, Roblee KJ (2009) Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York State. J Herp 43: 579–588.
37. Harris RN, James TY, Lauer A, Simon MA, Patel A (2006) Amphibian pathogen *Batrachochytrium dendrobatidis* is inhibited by the cutaneous bacteria of amphibian species. Ecohealth 3: 53–56.
38. Harris RN, Brucker RM, Walke JB, Becker MH, Schwantes CR, et al. (2009) Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. ISME J 3: 818–824.
39. Sheafor B, Davidson EW, Parr L, Rollins-Smith L (2008) Antimicrobial peptide defenses in the salamander, *Ambystoma tigrinum*, against emerging amphibian pathogens. J Wild Dis 44: 226–236.
40. Sorenson K (2004) Population characteristics of *Siren lacertina* and *Amphiuma means* in north Florida. Southeast Nat 3: 249–258.
41. Gehlbach FR, Kennedy SE (1978) Population ecology of a highly productive aquatic salamander (*Siren intermedia*). Southwest Nat 23: 423–430.
42. Frese PW, Mathis A, Wilkinson R (2003) Population characteristics, growth, and spatial activity of *Siren intermedia* in an intensively managed wetland. Southwest Nat 48: 534–542.
43. Hampton PM (2009) Ecology of the lesser siren, *Siren intermedia*, in an isolated eastern Texas pond. J Herp 43: 704–709.