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Prevalence of *Batrachochytrium dendrobatidis* and *B. salamandrivorans* in the Gulf Coast Waterdog, *Necturus beyeri*, from Southeast Louisiana, USA

The globally widespread amphibian fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has been linked to amphibian declines worldwide (Lips et al. 2006; Skerratt et al. 2007). In Louisiana, USA, *Bd* has been found in several amphibian species (Chatfield et al. 2012; Rothermel et al. 2008), but to our knowledge no population-level die-offs have been observed. Published literature on *Bd* prevalence in Louisiana is scant for some amphibian species and completely absent for many others. This trend is likely driven by the perception that *Bd* is not a major problem in this area due to a lack of observed die-offs attributable to chytridiomycosis.

Batrachochytrium salamandrivorans (*Bsal*) is an emerging amphibian fungal pathogen and was first described after populations of European Fire Salamanders (*Salamandra salamandra*) were decimated in the Netherlands (Martel et al. 2013). Since the observations in the Netherlands, *Bsal* has been found in wild salamander populations in neighboring Germany and Belgium (Spitzen-van der Sluijs et al. 2016), and has been linked to animals in captivity (Cunningham et al. 2015) and trade, with a proposed origin of Asia (Martel et al. 2014). Occurrence of *Bsal* has not been documented in any wild salamanders in North America, but due to the prolific trade in Asian salamanders, there is reason to think that it could arrive or may already be in North America. Prudent surveillance is vital, as a *Bsal* introduction in the United States could be devastating to this global hotspot of salamander diversity (Gray et al. 2015).

The Gulf Coast Waterdog, *Necturus beyeri*, is a permanently aquatic neotenic salamander that inhabits sandy spring-fed streams along the Gulf Coastal Plain, and its global conservation status is listed by NatureServe as 'apparently secure' (G4). In Louisiana, the Gulf Coast Waterdog is ranked S3 by the Louisiana Department of Wildlife and Fisheries, which indicates the species is rare and local throughout the state (21 to 100 known extant populations). Very little is known about prevalence rates of *Bd* in this species. Of two individuals examined by Chatfield et al. (2012), one tested positive for *Bd*. To our knowledge, this species has never been tested for *Bsal*, and no species of *Necturus* was used in challenge experiments by Martel et al. (2014). Therefore, it is unknown if members of this genus are susceptible to *Bsal* infection. Our aim was to conduct additional sampling for *Bd* and *Bsal* in *N. beveri* in Louisiana.

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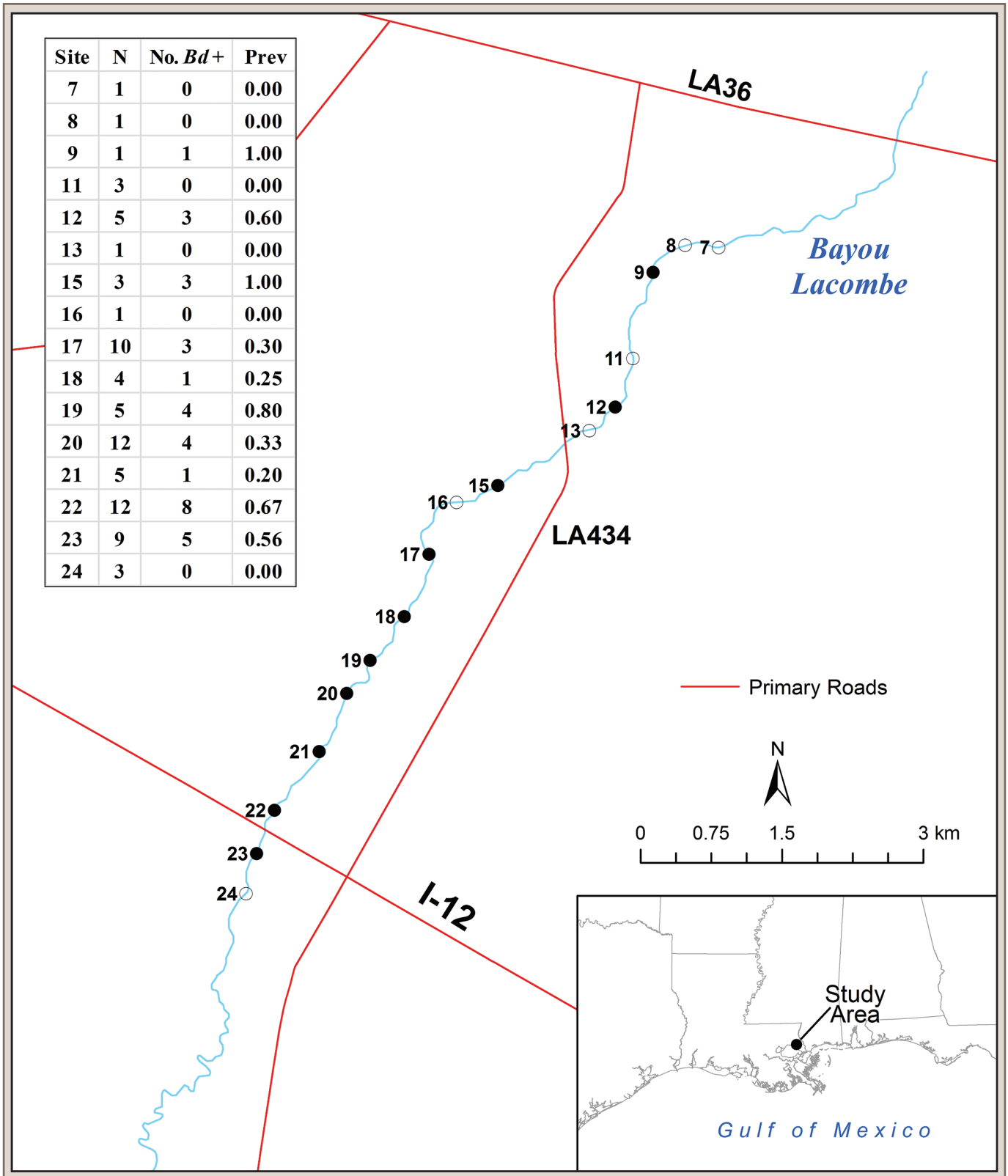
Gulf Coast Waterdogs were captured in southeast Louisiana with unbaited minnow traps at sites along ca. 12 km of Bayou Lacombe as part of an occupancy modelling study (Fig. 1). Although sampling took place over three weeks, all swabs used in this study were collected in one round of checking all traps and thus it is certain that each represents a unique individual. Here we report *Bd* and *Bsal* presence and pathogen load, as determined using quantitative polymerase chain reaction (PCR) results of swabs taken from 76 waterdogs captured 31 March through 2 April 2015 (Table 1). All captures were of live individuals and no other amphibians were captured in the traps.

To test for *Bd* and *Bsal* presence, waterdogs were placed in clear zip-top plastic bags and swabbed by gently rubbing a rayon-tipped swab (MWE113, Advantage Bundling SP, LLC, Durham, NC, USA) five times each on the following areas: the dorsum, each side, the venter, and the bottom of each foot. New nitrile gloves and new zip-top bags were used for each animal.

Genomic DNA was extracted from the swabs using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, CA, USA) and the manufacturer's recommended protocol for animal tissue with the following modifications: swabs were incubated for one hour and were vortexed and spun in a centrifuge after 30 minutes and again at the end of the incubation period; samples were eluted twice using 100 uL of elution buffer each time instead of 200 uL once. A multiplexed quantitative (real-time) PCR assay was used to detect the presence of *Bd* and *Bsal* DNA, following Boyle et al. (2004) and Blooi et al. (2013). Bovine serum albumin (0.7 mL) was added to each reaction well, as this has been shown to aid in overcoming problems with inhibition (Garland et al. 2010). All samples were run in triplicate and scored as positive if at least one replicate tested positive for *Bd* or *Bsal*. To confirm that reactions were not inhibited, an internal positive control (Applied Biosystems, Inc.) was added to one replicate of each sample (Hyatt et al. 2007). A seven-fold dilution series of plasmid standards from Pisces Molecular (Boulder, CO, USA) was run on every qPCR plate to enable quantification of pathogen load for both *Bd* and *Bsal*. As each assay used only 5 uL of the 200 uL of genomic DNA extracted, per assay load values were multiplied by 40 to estimate whole swab pathogen loads.

TABLE 1. Prevalence of *Batrachochytrium dendrobatidis* (*Bd*) infection by size (SVL = snout–vent length) and sex in *Necturus beyeri* in southeast Louisiana, USA.

	Sex	Size range (SVL, mm)	No. sampled	No. *Bd* positive	Prevalence
Juvenile	Unknown	63–83	7	2	0.29
Adult	Male	91–128	52	23	0.44
	Female	95–122	17	8	0.47
		Total	76	33	0.43



Site	N	No. *Bd*+	Prev
7	1	0	0.00
8	1	0	0.00
9	1	1	1.00
11	3	0	0.00
12	5	3	0.60
13	1	0	0.00
15	3	3	1.00
16	1	0	0.00
17	10	3	0.30
18	4	1	0.25
19	5	4	0.80
20	12	4	0.33
21	5	1	0.20
22	12	8	0.67
23	9	5	0.56
24	3	0	0.00

FIG. 1. Map of the study area showing the sites where *Necturus beyeri* were captured for assessment of infection with *Batrachochytrium dendrobatidis* (*Bd*) or *B. salmandrivorans* along Bayou Lacombe, St. Tammany Parish, Louisiana, USA. Solid circles represent sites where at least one individual tested *Bd*-positive (*Bd*+). Open circles represent sites where no individuals tested *Bd*-positive. The embedded table gives greater site-level detail: N = no. individuals sampled; Prev = *Bd* prevalence among individuals sampled at the site. The inset map indicates the location of the study area within Louisiana.

Overall, 33 of 76 (43%) waterdogs tested positive for *Bd* (Glorioso and Waddle 2017). Sixteen of 33 positive results were positive in all three runs, whereas the remaining 17 were positive in two of three runs. No difference in prevalence was observed by adult sex (Table 1). Eleven of 12 traps with three or more waterdogs captured and swabbed contained a mix of positive and negative results (Fig. 1). Pathogen loads were typically low in *Bd*-positive individuals (mean = 364, SD = 298; range 84–1212 plasmid equivalents). No waterdogs tested positive for *Bsal*, and no clinical signs of chytridiomycosis were observed in any captured individuals.

Our prevalence estimate for *Bd* in Gulf Coast Waterdogs was nearly twice the rate of all *Necturus* (N = 17) samples from Chatfield et al. (2012). The timing of our study coincided with the reported peak infection period found by others for semi-aquatic species in the southeast (e.g., Kinney et al. 2011; Rothermel et al. 2008). However, the timing of this study also coincides with the peak time to capture these animals, and captures of this species in mid-summer are rare. The lower *Bd* prevalence observed in Chatfield et al. (2012) may be attributable to warmer water temperatures experienced in summer by some of the permanently aquatic salamanders they studied (e.g., *Amphiuma* and *Siren*). Water temperatures where these animals live routinely exceed the optimal growth range of *Bd* (17°–25°C) in the hottest months in the Gulf Coastal Plain (Piotrowski et al. 2004).

The relatively cool stable environment that waterdogs inhabit suggests that they may serve as year-round hosts of *Bd* (Chatfield et al. 2012). Additionally, *Bd* has been shown to complete its life cycle in crawfish and transmit *Bd* infection to amphibians (McMahon et al. 2013). Crawfish were commonly captured in our traps and are a known primary prey item of Gulf Coast Waterdogs in at least some populations (Shoop and Gunning 1967). More work is needed examining the thermal relationship of *Bd*, crawfish, and Gulf Coast Waterdogs to clarify the possible year-round host hypothesis.

Currently it is not known if *Necturus* is susceptible to *Bsal*. Given that the optimal growth range of *Bsal* (10–15°C) is lower than that of *Bd* (Martel et al. 2013) and that the spring-fed lotic waters that *Necturus beyeri* inhabit provide suitable temperatures for *Bsal* for at least part of the year, there would be cause for concern if *Necturus beyeri* were shown to be susceptible to *Bsal*. More research into the effect of *Bd* on *Necturus* as well as increased monitoring for *Bsal* across the range of all taxa in the genus is warranted.

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