

A Preliminary Survey of *Batrachochytrium dendrobatidis* Exposure in Hellbenders from a Stream in Georgia, USA

Hellbenders (*Cryptobranchus alleganiensis*) are among the largest salamanders in the world. Hellbenders are thought to live over 25 years (Wheeler et al. 2003) and successful breeding populations are generally limited to relatively pristine stream systems. Hellbender populations appear to be declining due to a number of reasons, with habitat degradation such as siltation being a major factor (Briggler et al. 2007a). However, few studies have looked at pathogens as a potential cause of decline.

Batrachochytrium dendrobatidis (*Bd*) has been in the forefront of amphibian disease research in recent years (Duffus 2009). New reports of *Bd* range expansion and host diversity are now common in the literature (e.g., papers in *Herpetological Review*), but studies on the effect of *Bd* on salamander populations is limited, largely because of a lack of knowledge on the status of the populations prior to the introduction of *Bd*. In particular, there is a paucity of information regarding *Bd* in Georgia, USA (Rothermel et al. 2008; Timpe 2008), and few reports of *Bd* in Hellbenders are available: in Missouri and Arkansas, USA (Briggler et al. 2008); in Kentucky, USA (G. Lipps: data available at: www.spatalepidemiology.net/Bd-maps). The goal of our study was to obtain baseline data on *Bd* occurrence in a Hellbender population in Georgia.

Hellbenders were collected from Cooper Creek, Union County, Georgia, using a dip-net placed downstream of flipped rocks, as part of another study. Cooper Creek is a fairly swift, medium-sized, rocky-bottomed stream nestled within the Blue Ridge Mountains. Samples were collected opportunistically in March 2009, and in May 2009, additional samples were collected from a site upstream of the March samples. Each salamander was systematically swabbed 20 times along the ventral skin surface and feet with a sterile cotton swab. Samples were stored in individual cryovials, and frozen at -80° C until testing. Genomic DNA was extracted from each swab using the Qiagen

TABLE 1. *Batrachochytrium dendrobatidis* (*Bd*) occurrence in Hellbenders (*Cryptobranchus alleganiensis*) from Georgia, USA.

| Age Class | N | No. <i>Bd</i> -positive (%) | No. <i>Bd</i> -negative (%) | No. equivocal (%) |
|---------------|----|-----------------------------|-----------------------------|-------------------|
| March 2009 | | | | |
| Adults | 17 | 9 | 3 | 5 |
| Juveniles | 4 | 0 | 3 | 1 |
| Total | 21 | 9 (43) | 6 (28) | 6 (28) |
| May 2009 | | | | |
| Adults | 1 | 0 | 1 | 0 |
| Juveniles | 5 | 0 | 5 | 0 |
| Total | 6 | 0 (0) | 6 (100) | 0 (0) |
| Overall Total | 27 | 9 (33) | 12 (44) | 6 (22) |

DNA Purification Kit (Germantown, MD, USA) following the manufacturer's protocol and each sample was tested in duplicate for *Bd* by polymerase chain reaction (PCR) testing as described (Annis et al. 2004). A sample was only considered positive if both independent PCR reactions were positive; a single *Bd*-positive result was interpreted as being equivocal.

In total, 21 of 27 samples had unequivocal results, and 10 of 21 (48%) samples were *Bd*-positive (Table 1). More salamanders were *Bd*-positive during the March sampling period compared with May (Fisher's exact test, $p = 0.0152$); however, the low number of salamanders in the later sample ($N = 6$) may have been insufficient for determining an accurate *Bd* prevalence at that time. Skerratt et al. (2008) recommended sample sizes > 59 when *Bd* prevalence is expected to be low. During the March sampling period, more adults were positive compared with juveniles (Fisher's exact test, $p = 0.0357$), but again low sample sizes may have affected results (Table 1). Unfortunately, due to a freezer failure, the integrity of the 6 equivocal samples was compromised and they could not be retested.

Despite the small sample size of this study, we documented a high prevalence (48%) of *Bd*-positive Hellbenders. To our knowledge this is the first report of *Bd* in Hellbenders from Georgia, although *Bd* has been documented in captive Hellbenders (Briggler et al. 2007b) and in wild Hellbenders from five rivers in Missouri and Arkansas (Briggler et al. 2008), and in wild Hellbenders in Kentucky (G. Lipps, www.spatalepidemiology.net/Bd-maps). Further investigation is needed to determine the effects of *Bd* infection in Hellbenders. However, none of the salamanders that were sampled in our study exhibited clinical signs or had gross lesions suggestive of chytridiomycosis.

It is interesting to note that the samples obtained in March were collected in an area of the stream that is utilized heavily by humans for camping, fishing, and other recreational pursuits. In contrast, the May samples, which were all negative,

JESSICA L. GONYNOR*

MICHAEL J. YABSLEY

Warnell School of Forestry and Natural Resources and
Southeastern Cooperative Wildlife Disease Study
Department of Population Health, College of Veterinary Medicine
University of Georgia, Athens, Georgia 30602, USA

JOHN B. JENSEN

Nongame Conservation Section, Wildlife Resources Division
Georgia Department of Natural Resources, Forsyth, Georgia 31029, USA

* Corresponding author; e-mail: jgonynor@uga.edu

were from a relatively isolated site upstream. Additional studies along a gradient in the creek are needed to determine if the difference in *Bd* prevalence may be related to anthropogenic factors. Some species of ranids are common hosts for *Bd* and can serve as reservoirs (Sánchez et al. 2008; Daszak et al. 2004), so differences in amphibian communities between sites may affect prevalence. Additionally, there may be seasonal variation in the detection of *Bd* (Rothermel et al. 2008).

Recent studies on the morbidity and mortality of amphibians due to emerging infectious diseases, such as chytridiomycosis and *Ranavirus*, has highlighted the need to conduct surveillance for these pathogens in an effort to determine possible population effects (Duffus 2009). Although reports of these diseases in various species of salamanders are limited, they are increasing in number and geographic scope. The samples utilized for our study were collected by researchers who were conducting non-disease related research on these salamanders. We encourage others to take advantage of opportunities to collaborate with other researchers in order to carry out pathogen surveillance, particularly in at-risk cryptic species.

Acknowledgments.—We thank Rod Williams, Shem Unger, and other faculty and students from Purdue University for collecting most of the swabs from Hellbenders in March. Support was provided by the Federal Aid to Wildlife Restoration Act (50 Stat. 917) and through sponsorship from fish and wildlife agencies in Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia.

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