# PREVALENCE OF THE AMPHIBIAN PATHOGEN BATRACHOCHYTRIUM DENDROBATIDIS IN EASTERN HELLBENDERS (CRYPTOBRANCHUS A. ALLEGANIENSIS) IN WESTERN NORTH CAROLINA, USA

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Abstract.—Due to recent documented declines of Eastern Hellbender (Cryptobranchus a. alleganiensis) populations, in 2008–2012 we surveyed both wild-caught and captive hellbenders in Western North Carolina for the prevalence of a common amphibian pathogen, Batrachochytrium dendrobatidis (Bd). We sampled 165 wild and 15 captive animals of North Carolina origin. We collected morphometric data from hellbenders, noted the presence of any physical anomalies or injuries, and recorded water temperature and site elevation. We found Bd to be widespread in Western North Carolina waterways and in all river basins and sub-basins sampled. Batrachochytrium dendrobatidis prevalence was 27.9% for wild-caught animals and 26.7% for captives. Adult female hellbenders had a significantly higher prevalence of Bd (38.8%) than adult males (19.7%; P = 0.0207). All age classes tested positive for Bd, including gilled larvae. From examining hellbender body condition, we found no physical evidence of acute infection or compromised immunity due to disease. There was no significant relationship between the presence of Bd and physical injuries or anomalies (P = 0.1196). We also found that water temperature (P = 0.5038) and elevation (P = 0.5100) were not significant for predicting the presence of Bd. Ours is the first report of Bd for Eastern Hellbenders in North Carolina. Although it does not appear that hellbenders in North Carolina are in crisis due to disease, future monitoring and surveillance efforts within populations should continue, particularly across a variety of habitat disturbance regimes.

Key Words.—amphibian disease; body condition; chytrid; hellbenders; environmental conditions

#### INTRODUCTION

Numerous anuran and caudate studies in the Eastern United States have documented the presence of the amphibian pathogen Batrachochytrium dendrobatidis (Bd), an aquatic fungus that causes chytridiomycosis, a severe skin disease (e.g., Grant et al. 2008; Rothermel et al. 2008; Pullen et al. 2010; Hill et al. 2011; Huang and Wilson 2013). In the Southern Appalachian Mountain region of Western North Carolina and Eastern Tennessee, Bd has either been detected at low prevalence (< 1-6%; Chatfield et al. 2009; Chinnadurai et al. 2009; Caruso and Lips 2012; Kiemnec-Tyburczy et al. 2012; Rothermel et al. 2013) or not at all in a variety of terrestrial and aquatic amphibian habitats and species (Hossack et al. 2010; Keitzer et al. 2011; Moffitt 2012). Although few amphibian mortality events have been linked to the pathogen in the Southern Appalachians, disease surveillance efforts for the majority of the region's diverse salamander species, particularly those in large riverine systems, are lacking.

Disease could be one of many factors affecting the hellbender (*Cryptobranchus alleganiensis*), a giant, riverine salamander, one of the more imperiled salamanders in the world (IUCN. 2013. IUCN Red List

of Threatened Species. Available from <a href="http://www.iucn">http://www.iucn</a> redlist.org [Accessed 3 December 2013]). Hellbenders have declined dramatically throughout their historic range, particularly in recent decades (Mayasich et al. 2003). Population declines have been documented for Ozark Hellbender (Cryptobranchus a. bishopi) in Missouri and both Ozark and Eastern Hellbender (Cryptobranchus a. alleganiensis) in Arkansas (Trauth et al. 1992; Wheeler et al. 2003; Nickerson and Briggler 2007; Hiler et al. 2013). Other areas of documented Eastern Hellbender decline include the Tennessee River drainage in Alabama (Graham et al. 2011), the Ohio River drainage of Indiana (Burgmeier et al. 2011a; Olson et al. 2013), Ohio (Pfingsten 1990), West Virginia (Keitzer et al. 2013), and Maryland (Gates et al. 1985), the upper Allegheny River drainage of New York (Foster et al. 2009), and the Susquehanna River drainage in Maryland (Gates et al. 1985) and New York (Quinn et al. 2013). Hellbender populations are under threat from multiple factors including habitat degradation, habitat and natural systems disturbance (Williams et al. 1981; Mayasich et al. 2003; Phillips and Humphries 2005; Briggler et al. 2007b), overharvesting and overcollecting (Nickerson and Briggler 2007), toxins and

2007; Hopkins and DuRant 2011; Unger et al. 2013a).

Of the numerous threats to hellbenders, disease is one of the more poorly understood, and researchers are just beginning to document the scope and severity of the Initial work indicates Bd may be pathogen Bd. widespread in aquatic systems but may have a low prevalence in some headwater streams and protected drainages (Timpe et al. 2008; Hossack et al. 2010; Gratwicke et al. 2011), or some salamanders occupying streams in upper watersheds might have the ability to live with these pathogens at sub-clinical levels (Chinnadurai et al. 2009; Vazquez et al. 2009). Prior to 1969, there was no evidence that Bd was present in Ozark Hellbenders (Bodinof et al. 2011), suggesting the exotic fungal pathogen was introduced several decades ago but has since become well established. Genetic analysis of Bd found on Ozark and Eastern Hellbenders revealed the same fungal types that are found on amphibians around the world, providing more evidence that Bd is a novel pathogen in the United States that has spread widely and invasively since its introduction (Tominaga et al. 2013).

Batrachochytrium dendrobatidis in cryptobranchids has been documented in many locations. The fungus has been found on the Japanese Giant Salamander (Andrias japonica; Goka et al. 2009), in captive populations of both Ozark and Eastern Hellbenders in Missouri (Briggler et al. 2007a), and in wild hellbenders in individual watersheds or streams in numerous states (e.g., Briggler et al. 2008; Burgmeier et al. 2011b; Gonynor et al. 2011; Regester et al. 2012; Souza et al. 2012). However, our study is the first widespread disease surveillance effort for Eastern Hellbender at the state level and the first in North Carolina. In this project, our main objectives were to examine the prevalence and distribution of Bd in hellbenders in Western North Carolina, sampling within a variety of water temperatures and elevations, to assess hellbender body condition looking for any visible signs of disease, and to establish baselines for future surveillance and monitoring.

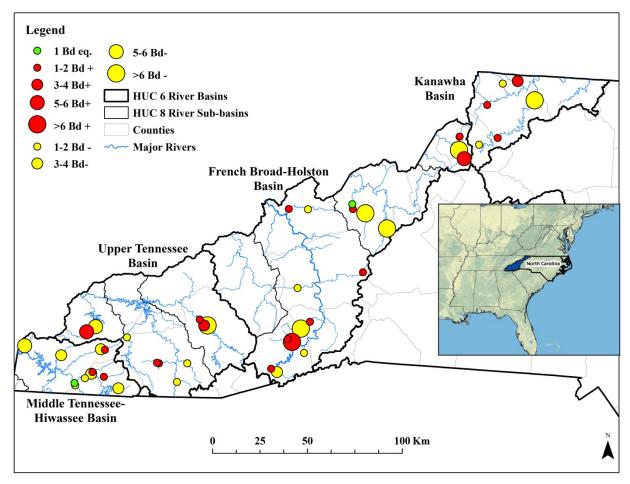
#### MATERIALS AND METHODS

From 2008-2012, primarily in the months of May-August, we collected 165 Bd samples from wild populations of Eastern Hellbenders in Western North Carolina (n = 57 sites). Samples came from 33 individual river systems in 13 counties, eight river subbasins (HUC 8), and across all four river basins (HUC 6), where hellbenders occur: the Kanawha, the French Broad-Holston, the Upper Tennessee, and the Middle Tennessee-Hiwassee Basins. All watersheds surveyed drain to the Tennessee or Ohio Rivers, then the Mississippi River, and eventually to the Gulf of Mexico.

pollutants, and possibly disease as well (Solis et al. Major waterways in these basins in Western North Carolina include the Hiwassee, Little Tennessee, Tuckasegee, Pigeon, French Broad, Nolichucky, Watauga, and New Rivers. Due to the sensitivity of capture locations and the threat of site disturbance and illegal harassment or collection of hellbenders, a protected, Special Concern Species in North Carolina (North Carolina Wildlife Resources Commission 2005). we have withheld specific site details on file with the North Carolina Wildlife Resources Commission.

> We collected skin swab samples for disease surveillance opportunistically and concurrent with ongoing hellbender population monitoring, inventories, and research from May through August each year. Hellbender surveys consisted of snorkeling and visual encounter techniques (Nickerson and Krysko 2003; Browne et al. 2011). We recorded morphometric data, age class, and sex of captured hellbenders and noted any visible wounds, scars, injuries, or anomalies as part of routine data collection methods. We laundered mesh bags used for holding, handling, and processing hellbenders after use in hot water, detergent, and diluted bleach. Unless hellbender secondary sex characteristics were present, as a general rule, we considered animals with total lengths less than 25 cm to be juveniles; however, suggested minimum adult sizes vary widely in the literature from approximately 20 cm up to 39 cm total length (e.g., Nickerson and Mays 1973; Taber et al. 1975; Peterson et al. 1983; Hecht-Kardasz et al. 2012; Pugh et al. 2013). At all capture locations, we used a standard bulb thermometer or a dissolved oxygen and temperature meter to record water temperature and a Garmin<sup>™</sup> 60CSx Global Positioning System (GPS; Garmin International, Inc, Olathe, Kansas, USA) unit to record latitude and longitude coordinates and an estimate of site elevation.

> Batrachochytrium dendrobatidis has been documented in captive hellbenders (Briggler et al. 2007a), so we tested 15 captive hellbenders (six adults, nine iuveniles) of North Carolina origin, all from the French Broad-Holston River Basin. At the time of swab collections, these animals were housed at the Como Park Zoo (St. Paul, Minnesota, USA), the North Carolina Zoological Park (Asheboro, North Carolina, USA), the North Carolina Wildlife Resources Commission's Marion Fish Hatchery and Aquaculture Center (Marion, North Carolina, USA), the North Carolina Wildlife Resources Commission's Pisgah Wildlife Education Center (Pisgah Forest, North Carolina, USA), and Earthshine Nature Programs (Lake Toxaway, North Carolina, USA). The Amphibian Disease Laboratory at the San Diego Zoo's Institute for Conservation Research (Escondido, California, USA) offered sampling protocol and supplies for collecting Bd swabs in the field. We used sterile, plastic-handled, fine-tipped, rayon swabs (Dryswab TM) from Advantage Bundling SP (catalog number MW113,



**FIGURE 1.** Number of *Batrachochytrium dendrobatidis* equivocal (*Bd* eq.), positive (*Bd*+), and negative (*Bd*-) samples from wild-caught Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) per individual stream across all HUC 6 river basins in Western North Carolina.

Medical Wire & Equipment Co., Corsham, Wiltshire, England) and screw-top, 2.0 mL plastic vials. For *Bd* samples, we used a single swab in approximately 20 passes along the skin on the ventral surface of hellbenders, focusing around the pelvis, inner thighs, foot pads, and between the toes (Brem et al. 2007; Gonynor et al. 2011; Regester et al. 2012; Souza et al. 2012). We cut swabs 2–3 cm from the bottom and stored them dry in individually labeled vials kept in a cooler until they could be transferred to a freezer for longer storage throughout the field season.

Each fall, we shipped the frozen vials overnight on dry ice to The Amphibian Disease Laboratory at the San Diego Zoo's Institute for Conservation Research for testing. Lab staff conducted *Bd* assays with isolated DNA on a prepared template using Prep-Man® Ultra Sample Preparation Agent (catalog number 4318930, Applied Biosystems, Foster City, California, USA) for use in TaqMan® Real-Time Polymerase Chain Reaction (PCR) procedures (Skerratt et al. 2011) based on published protocol (Annis et al. 2004; Boyle et al. 2004; Hyatt et al. 2007) and used with similar amphibian disease surveillance studies (Keitzer et al. 2011; Souza et

al. 2012). Diluted, cultured *Bd* zoospores were used for positive controls, and reactions were run in triplicate (Keitzer et al. 2011; Souza et al. 2012).

We used Epi Info<sup>TM</sup> statistical software (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) to calculate summary statistics of percent prevalence, with 95% confidence intervals, as demonstrated by Souza et al. (2012). We used included Pearson's Chisquared or Fisher's exact test to examine differences in Bd prevalence among river basins, hellbender age classes, and hellbender sexes. We also used Pearson's Chi-squared or Fisher's exact test to investigate differences in the frequency of physical injuries or Bd-positive versus anomalies Bd-negative hellbenders, all samples pooled, and across sexes, age classes, and river basins. We tested normality of site elevation and temperature data with a Shapiro-Wilk goodness-of-fit test and used ANOVA and logistic regression to analyze the relationship between those metrics for sites where Bd was detected. Statistical analyses were conducted using Rcmdr package, v.2.0.3 in R. v.3.0.2 statistical software (The R Foundation for Statistical Computing, Vienna, Austria) at P < 0.05.

#### RESULTS

We found Bd to be widespread among North Carolina's hellbender populations. From 2008–2012, we found Bd in all HUC 6 river basins sampled (n = 4), in all HUC 8 sub-basins sampled (n = 8), in 12 of 13 (92.3%) counties sampled, and in 19 of 33 (57.6%) individual stream systems sampled (Table 1, Fig. 1). Where zoospore equivalent data were available for Bd-positive samples (n = 38), zoospore counts across replicates averaged 12 with a maximum of 380 zoospores/ $\mu$ L. A majority of positive samples (52.6%) had average zoospore equivalents < 1, 34.2% had an average of 1–20, and 13.2% averaged > 20 zoospores/ $\mu$ L.

Of the 19 Bd-positive streams, 13 (68.4%) had both negative and positive Bd samples, while six (31.6%) only had positive Bd samples with no negative or equivocal results. Of 165 samples total, 46 (27.9%) were positive for Bd, 117 (70.9%) negative, and two (1.2%) equivocal. The equivocal results occurred in adult hellbenders from two different streams, one where all other samples were negative and one that had both positive and negative results.

Of the 140 adults sampled, 40 (28.6%) were positive for Bd, while six (24.0%) of the 25 juveniles sampled were positive, including two gilled larvae (Table 2). Prevalence of Bd between adults and juveniles was not significantly different (Fisher's exact test, P = 0.8096). Not including adult hellbenders whose sex was undetermined in the field, the number of adult males and females tested was almost identical (66M, 67F). However, females showed a significantly higher prevalence of Bd than males, with 26 females (38.8%) positive compared to 13 males (19.7%;  $\chi^2 = 5.35$ , df = 1, P = 0.021; Table 2). Hellbenders from the Kanawha River Basin showed the highest prevalence of Bd (35.3%), while the Middle Tennessee-Hiwassee River Basin had the lowest (18.5%; Table 2). There were no significant differences in Bd prevalence among river basins ( $\chi^2 = 1.90$ , df = 3, P = 0.593). The 15 captive hellbenders of North Carolina origin we tested showed a similar prevalence of Bd as did wild populations. Two of six adults and two of nine juveniles tested positive for Bd for a combined prevalence of 26.7% (95% C.I. = 7.8– 55.1%).

None of the hellbenders tested for *Bd* showed any noticeable physical or behavioral symptoms that would indicate severe skin infection, lethargy, emaciation, morbidity, or other evidence of compromised immunology or outright disease. Overall we noted only minor abrasions, head, body, or tail scarring, healed or healing wounds, and regenerating tissue (tail tips, digits, limbs; see Appendix A). Not counting one *Bd*-negative animal whose body condition was not recorded, 46.9%

of all captures had some type of minor injury or former wound. Of the animals that tested negative for Bd, 50.9% had minor physical anomalies compared to 37.0% of animals positive for Bd. On very few occasions did we note the presence of suspicious-looking tissue such as inflamed masses or a white-colored fungus at the site of an old wound. Subsequent laboratory tests at the North Carolina Zoological Park determined the whitecolored coating to be a common type of fish fungus in the genus Saprolegnia. There were no significant differences in the frequency of physical injuries or anomalies in Bd-positive hellbenders versus Bd-negative hellbenders (Fisher's exact test, P = 0.120) or in adult males versus adult females (Fisher's exact test, P =There were no significant differences in 0.116). hellbender physical condition across river basins ( $\chi^2$  = 2.769, df = 3, P = 0.429). However, as to be expected, there were significantly fewer injuries or anomalies in juveniles compared to adults (Fisher's exact test, P <0.001).

Site elevations and water temperatures, collected at the time of sampling, were normally distributed (W = 0.969, P = 0.303; W = 0.978, P = 0.705, respectively). Elevations ranged from 361 m to 960 m (mean = 658.3 ± 136.4 m), and water temperatures ranged from 12.8° C to 27.8° C (mean =  $20.7 \pm 3.1$ ° C). There was a significant, inverse relationship between site elevation and water temperature, as would be expected with typically cooler temperatures at higher elevations ( $F_{1,43} = 6.61$ ,  $P_{-} = 0.014$ ). However, neither water temperature ( $r^2 = 0.007$ , df = 1, P = 0.504) nor elevation ( $r^2 = 0.006$ , df = 1,  $P_{-} = 0.510$ ) were significant for predicting the presence of Bd at field sites.

#### DISCUSSION

Results of our disease surveillance in hellbenders reiterate what similar studies have found, that Bd may be well established and widespread in aquatic systems and can be detected in both juvenile and adult hellbenders (Regester et al. 2012; Souza et al. 2012). We also detected Bd in two gilled hellbender larvae. This finding is contrary to a previous study (Keitzer et al. 2011) suggesting that larval aquatic salamanders may not be susceptible to Bd due to the limited amount of epidermal keratin. The overall Bd prevalence we documented for pooled samples across all river basins was 27.9%, which was comparable to the 26% prevalence (Souza et al. 2012) and within the range of 16-50% prevalence (Tominaga et al. 2013) reported in East Tennessee across multiple watersheds shared with Western North Carolina. Our results show a lower prevalence than what was found in Pennsylvania's hellbenders across two drainages, 43% (Regester et al. 2012), or in Georgia's hellbenders in one stream across early and late spring sampling seasons, 48% (Gonynor et al. 2011).

**TABLE 1.** Number (percentage frequency [# detected/total], 95% confidence interval) of individual Eastern Hellbender (*Cryptobranchus a. alleganiensis*) streams where *Batrachochytrium dendrobatidis* (*Bd*) was detected in at least one sample (*Bd*+) vs. streams with no detection (*Bd*-), per HUC 6 river basin in Western North Carolina, from 2008–2012.

HUC 6 River Basin	n	No. Streams <i>Bd</i> +	No. Streams <i>Bd</i> -	
Middle Tennessee-Hiwassee	8	3 (37.5; 8.5–75.5)	5 (62.5°; 24.5–91.5)	
Upper Tennessee	8	5 (62.5; 24.5–91.5)	3 (37.5; 8.5–75.5)	
French Broad-Holston	12	8 (66.7 <sup>6</sup> ; 34.9–90.1)	4 (33.3; 9.9–65.1)	
Kanawha	5	3 (60.0; 14.7–94.7)	2 (40.0; 5.27–85.34)	
Total	33	19 (57.6; 39.22–74.52)	14 (42.4; 25.5–60.8)	

<sup>&</sup>lt;sup>a</sup>one equivocal result occurred in a stream with only negative results otherwise.

Assays of *Bd* in Florida, Mississippi, and Louisiana for other large-bodied, fully aquatic salamanders such as *Amphiuma* sp., *Necturus* sp., *Pseudobranchus* sp., and *Siren* sp. showed a similar infection prevalence of 34% (Chatfield et al. 2012), but in a separate study in Florida, *Bd* was not detected in *Siren* sp. or *Amphiuma* sp. (Rizkalla 2010).

Despite a smaller sample size than that of wild-caught animals in North Carolina, the 26.7% prevalence of Bd in captive hellbenders was relatively the same. result supports other studies that indicate hellbenders raised in captivity are also susceptible to Bd infection, as reported for captive Ozark and Eastern Hellbenders in Missouri (Briggler et al. 2007a). Our 2 y-old juveniles, hatched in captivity at Ft. Worth Zoo, originated as eggs collected from a North Carolina river known to be positive for Bd. These animals were Bd-negative upon arrival back to North Carolina at age 20 mo. When swabbed again for Bd, six months after arrival to the fish hatchery, two were Bd-positive but apparently experiencing a sub-clinical level of infection. It is unknown when these captive animals contracted Bd, but it is possible the fungus could be in the hatchery's water system that includes spring-fed a Batrachochytrium dendrobatidis has been documented in fish hatcheries and natural or artificial ponds throughout the Eastern United States (e.g., Green and Dodd 2007; Rothermel et al. 2008; Groner and Relyea 2010; Goodman and Ararso 2012; Bletz and Harris 2013), so it is likely the captive juveniles contracted the fungus once housed at the fish hatchery. surveillance of captive stock and baseline assays of Bd in resident pond-dwelling amphibians at the hatchery may reveal whether these pathogens pose a serious health risk to the captive hellbenders in this setting.

Hellbenders positive for *Bd* in North Carolina appeared to have no clinical symptoms or indications of full-blown disease, nor did they show signs of poor immunity or excessive stress that might make them more susceptible to illness (Tominaga et al. 2013). Rather, our animals seemed to be living with low-level fungal infections and likely have been doing so for some time. For positive samples, zoospore equivalents were quite

compared to thresholds established low chytridiomycosis in ranid frogs (> 10,000 zoospores/µL; Vrendenburg et al. 2010; Kinney et al. 2011), demonstrating that hellbenders were not suffering from outright disease despite possessing sub-clinical infections of Bd. The physical conditions we observed are commonly seen in hellbenders and can often be attributed to interactions with each other and predators (Nickerson and Mays 1973; Miller and Miller 2005). We did not see similar gross physical abnormalities, severe fungal infections, unhealed wounds, tumors, lesions, or other signs of poor immunity that have been reported for declining Ozark hellbender populations (Wheeler et al. 2002; Hiler et al. 2005). observations of physical condition were consistent with those of other regional hellbender disease surveillance efforts and not indicative of chytridiomycosis (Gonynor et al. 2011; Regester et al. 2012; Souza et al. 2012).

The significantly higher prevalence of Bd in adult female hellbenders (38.8%) compared to adult males (19.7%) in our results should be explored in future surveillance efforts and with larger sample sizes. Despite a similar prevalence of physical injuries or anomalies between males and females, future studies should examine whether differences among male and female activity levels and interactions with other hellbenders or predators have any effect on the amount or type of cutaneous bacteria available for disease resistance, or whether life-history strategies, movement patterns, or home range sizes may increase the exposure pathogens, females to increasing susceptibility. If differential infection rates exist between males and females, then a greater risk of female mortality may have broad implications for declining population health, structure, and reproductive success (Unger et al. 2013b).

Further, Grant et al. (2008) suggested a link between frequency of skin shedding and disease resistance in aquatic, desmognathine salamanders, and Meyer et al. (2012) examined the role of skin sloughing to reduce fungal spores and microbes in Cane Toads (*Rhinella marina*). Nickerson et al. (2011) studied the suite of microbes available on hellbender skin that aid in tissue

bone equivocal result occurred in a stream with both positive and negative results.

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**TABLE 2.** Number (percentage frequency [# detected/total], 95% confidence interval) of *Batrachochytrium dendrobatidis* positive (*Bd*+), negative (*Bd*-), and equivocal (*Bd* eq.) samples from wild-caught Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) by age class and sex, per HUC 6 river basin in Western North Carolina, 2008–2012.

Age Class / Sex	n	Bd+	Bd-	Bd eq.
Middle Tennessee-Hiwassee				
Adults	24	5 (20.8; 7.1–42.2)	18 (75.0; 53.3–90.2)	1 (4.17; 0.1–21.1)
Males	11	3 (27.3; 6.0–61.0)	7 (63.6; 30.8–89.1)	1 (9.1; 0.2–41.3)
Females	12	2 (16.7; 2.1–48.4)	10 (83.3; 51.6–97.9)	0
Unknown	1	0	1 (100)	0
Juveniles	3	0	3 (100)	0
Basin Total	27	5 (18.5; 6.3–38.1)	21 (77.8; 57.7–91.4)	1 (3.7; 0.1–19.0)
Upper Tennessee				
Adults	32	11 (34.4; 18.6–53.2)	21 (65.6; 46.8–81.4)	0
Males	14	3 (21.4; 4.7–50.1)	11 (78.6; 49.2–95.3)	0
Females	18	8 (44.4; 21.5–69.2)	10 (55.6; 30.8–78.5)	0
Unknown	0	0	0	0
Juveniles	1	0	1 (100)	0
Basin Total	33	11 (33.3; 18.0–51.8)	22 (66.7; 48.2–82.0)	0
French Broad-Holston				
Adults	68	18 (26.5; 16.5–38.6)	49 (72.1; 59.9–82.3)	1 (1.5; 0.0–7.9)
Males	34	5 (14.7; 5.0–31.1)	28 (82.4; 65.5–93.2)	1 (2.9; 0.1–15.3)
Females	28	12 (42.9; 24.5–62.8)	16 (57.1; 37.2–75.5)	0
Unknown	6	1 (16.7; 0.4–64.1)	5 (83.3; 35.9–99.6)	0
Juveniles	20	6 (30.0; 11.9–54.3)	14 (70.0; 45.7–88.1)	0
Basin Total	88	24 (27.3; 18.3–37.8)	63 (71.6; 61.0–80.1)	1 (1.1; 0.0–6.2)
Kanawha				
Adults	16	6 (37.5; 15.2–64.6)	10 (62.5; 35.4–84.8)	0
Males	7	2 (28.6; 3.7–71.0)	5 (71.4; 29.0–96.3)	0
Females	9	4 (44.4; 13.7–78.8)	5 (55.6; 21.2–86.3)	0
Unknown	0	0	0	0
Juveniles	1	0	1 (100)	0
Basin Total	17	6 (35.3; 14.2–61.7)	11 (64.7; 38.3–85.8)	0
TOTALS				
Adults	140	40 (28.6; 21.3–36.8)	98 (70.0; 61.7–77.5)	2 (1.43; 0.2–5.1)
Males	66	13 (19.7; 10.9–31.3)	51 (77.3; 65.3–86.7)	2 (3.00; 0.4–10.5)
Females	67	26 (38.8; 27.1–51.5)	41 (61.2; 48.5–72.9)	0
Unknown	7	1 (14.3; 0.4–57.9)	6 (85.7; 42.1–99.6)	0
Juveniles	25	6 (24.0; 9.4–45.1)	19 (76.0; 54.9–90.6)	0
Overall Total	165	46 (27.9; 21.2–35.4)	117 (70.9; 63.3–77.7)	2 (1.20; 0.2–4.3)

regeneration and combat infection, but the direct role of hellbender skin and slime components as a natural barrier and defense to pathogens like *Bd* remains largely unknown (Nickerson and Mays 1973; Mayasich et al. 2003). The relationship between activity levels and behaviors, frequency of skin shedding (or slime production), and natural disease resistance has not been adequately studied in hellbenders.

We found no significant relationship between site elevation and Bd presence or between water temperature and Bd presence, despite the fact that a majority of sampled sites fell within the temperature threshold most optimal for Bd growth, 17–25° C (Daszak et al. 2003; Piotrowski et al. 2004). Fisher et al. (2009) discussed the potential increased risk of Bd for sites with cooler temperatures and higher altitudes but noted that many studies have detected Bd in low-elevation, warmer

locations as well. *Batrachochytrium dendrobatidis* can be rare or occur in low prevalence in cooler, headwater streams (Hossack et al. 2010), but the effect of seasonality on *Bd* prevalence in these cooler, high-elevation habitats has not been examined thoroughly (Chatfield et al. 2012). However, differential prevalence and virulence of *Bd* has been documented across seasons in a variety of other habitats, with fungal loads increasing in the spring and early summer (Kriger and Hero 2007; Kinney et al. 2011; Lannoo et al. 2011) or decreasing after episodic, dramatic environmental change such as drought (Terrell et al. 2014). These factors have not been examined in North Carolina, particularly in riverine systems.

Although this study provided a baseline of *Bd* prevalence in North Carolina's hellbenders, researchers must continue to monitor population health long-term,

given the reality that the fungus is widespread and, by now, likely well established in hellbender waterways. A long-term disease surveillance effort at the population level and across a variety of river habitat conditions, landscape positions, elevations, and seasons may help us understand what factors or stressors could exacerbate the spread and virulence of this pathogen. These disease dynamics could ultimately affect hellbender populations and influence conservation efforts in North Carolina and throughout the Southern Appalachian region.

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#### LITERATURE CITED

- Annis, S.L., F.P. Dastoor, H. Ziel, P. Daszak, and J.E. Longcore. 2004. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. Journal of Wildlife Diseases 40:420–428.
- Bletz, M.C., and R.N. Harris. 2013. Occurrence of *Batrachochytrium dendrobatidis* in *Notophthalmus viridescens* in northwestern Virginia, USA. Herpetological Review 44:257–259.
- Bodinof, C.M., J.T. Briggler, M.C. Duncan, J. Beringer, and J. Millspaugh. 2011. Historic occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in Hellbender *Cryptobranchus alleganiensis* populations from Missouri. Diseases of Aquatic Organisms 96:1–7.

- Boyle, D.G., D.B. Boyle, V. Olsen, J.A.T. Morgan, and A.D. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms 60:141–148.
- Brem, F., J.R. Mendelson, III, and K.R. Lips. 2007. Field-sampling protocol for *Batrachochytrium dendrobatidis* from living amphibians, using alcohol preserved swabs. Version 1.0. Conservation International, Arlington, Virginia, USA. Available from <a href="http://www.amphibians.org">http://www.amphibians.org</a> [Accessed 9 December 2013].
- Briggler, J.T., K.A. Larson, and K.J. Irwin. 2008. Presence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) on Hellbenders (*Cryptobranchus alleganiensis*) in the Ozark Highlands. Herpetological Review 39:443–444.
- Briggler, J.T., J. Ettling, M. Wanner, C. Schuette, M. Duncan, and K. Goellner. 2007a. *Cryptobranchus alleganiensis* (Hellbender). Chytrid fungus. Herpetological Review 38:174.
- Briggler, J.T., J. Utrip, C. Davidson, J. Humphries, J. Groves, T. Johnson, J. Ettling, M. Wanner, K. Traylor-Holzer, D. Reed, et al. (Eds.). 2007b. Hellbender population and habitat viability assessment: final report. IUCN/ SSC Conservation Specialist Group, Apple Valley, Minnesota, USA.
- Browne, R.K., H. Li, D. McGinnity, S. Okada, W. Zhenghuan, C.M. Bodinof, K.J. Irwin, A. McMillan, and J.T. Briggler. 2011. Survey techniques for giant salamanders and other aquatic Caudata. Amphibian and Reptile Conservation 5:1–16.
- Burgmeier, N.G., S.D. Unger, T.M. Sutton, and R.N. Williams. 2011a. Population status of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Journal of Herpetology 45:195–201.
- Burgmeier, N.G., S.D. Unger, J.L. Meyer, T.M. Sutton, and R.N. Williams. 2011b. Health and habitat quality assessment for the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana, USA. Journal of Wildlife Diseases 47:836–848.
- Caruso, N.M., and K.R. Lips. 2012. Truly enigmatic declines in terrestrial salamander populations in Great Smoky Mountains National Park. Diversity and Distributions 2012:1–11.
- Chatfield, M.W., P. Moler, and C.L. Richards-Zawaki. 2012. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, in fully aquatic salamanders from Southeastern North America. PLoS ONE 7:e44821. doi:10.1371/journal.pone.0044821.
- Chatfield, M.W., B.B. Rothermel, C.S. Brooks, and J.B. Kay. 2009. Detection of *Batrachochytrium dendrobatidis* in amphibians from the Great Smoky

- Mountains of North Carolina and Tennessee, USA. Herpetological Review 40:176–179.
- Chinnadurai, S.K., D. Cooper, D.S. Dombrowski, M.F. Poore, and M.G. Levy. 2009. Experimental infection of native North Carolina salamanders with *Batrachochytrium dendrobatidis*. Journal of Wildlife Diseases 45:631–636.
- Daszak, P., A.A. Cunningham, and A.D. Hyatt. 2003. Infectious disease and amphibian population declines. Diversity and Distributions 9:141–150.
- Fisher, M.C., T.W.J. Garner, and S.F. Walker. 2009. Global emergence of *Bd* and amphibian chytridiomycosis in space, time, and host. Annual Review of Microbiology 63:291–310.
- Foster, R.L., A.M. McMillan, and K.J. Roblee. 2009. Population status of Hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York State. Journal of Herpetology 43:579–588.
- Gates, J.E., C.H. Hocutt, J.R. Stauffer, Jr., and G.J. Taylor. 1985. The distribution and status of *Cryptobranchus alleganiensis* in Maryland. Herpetological Review 16:17–18.
- Goka, K., J. Yokoyama, Y. Une, T. Kuroki, K. Suzuki, M. Nakahara, A. Kobayashi, S. Inaba, T. Mizutani, and A.D. Hyatt. 2009. Amphibian chytridiomycosis in Japan: distribution, haplotypes, and possible route of entry into Japan. Molecular Ecology 18:4757–4774.
- Gonynor, J.A., M.J. Yabsley, and J.B. Jensen. 2011. A preliminary survey of *Batrachochytrium dendrobatidis* exposure in Hellbenders from a stream in Georgia, USA. Herpetological Review 42:58–59.
- Goodman, R.M., and Y.T. Ararso. 2012. Survey of Ranavirus and the fungus *Batrachochytrium* in frogs in Central Virginia, USA. Herpetological Review 43:78–80.
- Graham, S.P., E.C. Soehren, G.R. Cline, C.M. Schmidt, W.B. Sutton, J.R. Rayburn, S.H. Stiles, and J.A. Stiles. 2011. Conservation status of Hellbenders (*Cryptobranchus alleganiensis*) in Alabama, USA. Herpetological Conservation and Biology 6:242–249.
- Grant, E.H.C., L.L. Bailey, J.L. Ware, and K.L. Duncan. 2008. Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in stream and wetland amphibians in Maryland, USA. Applied Herpetology 5:233–241.
- Gratwicke, B., M. Evans, E.H.C. Grant, J. Greathouse, W.J. McShea, N. Rotzel, and R.C. Fleischer. 2011. Low prevalence of *Batrachochytrium dendrobatidis* detected in Appalachian salamanders from Warren County, Virginia, USA. Herpetological Review 42:217–219.
- Green, D.E., and C.K. Dodd, Jr. 2007. Presence of amphibian chytrid fungus *Batrachochytrium dendrobatidis* and other amphibian pathogens at warm-water fish hatcheries in Southeastern North

- America. Herpetological Conservation and Biology 2:43–47.
- Groner, M.L., and R.A. Relyea. 2010. *Batrachochytrium dendrobatidis* is present in northwest Pennsylvania, USA, with high prevalence in *Notophthalmus viridescens*. Herpetological Review 41:462–465.
- Hecht-Kardasz, K.A., M.A. Nickerson, M. Freake, and P. Colclough. 2012. Population structure of the Hellbender (*Cryptobranchus alleganiensis*) in a Great Smoky Mountains stream. Bulletin of the Florida Museum of Natural History 51:227–241.
- Hiler, W.R., B.A. Wheeler, and S.E. Trauth. 2005. Abnormalities in the Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*) in Arkansas: a comparison between two rivers with a historical perspective. Journal of the Arkansas Academy of Science 59:88–94.
- Hiler, W.R., B.A. Wheeler, and S.E. Trauth. 2013. The decline of the Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*) in the Spring River, Arkansas, USA. Herpetological Conservation and Biology 8:114–121.
- Hill, R.L, M.G. Levy, E.K. Timpe, and J.B. Kaylock. 2011. Additional reports of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* from Georgia, USA. Herpetological Review 42:376–378.
- Hopkins, W.A., and S.E. DuRant. 2011. Innate immunity and stress physiology of Eastern Hellbenders (*Cryptobranchus alleganiensis*) from two stream reaches with differing habitat quality. General and Comparative Endocrinology 174:107–115.
- Hossack, B.R., M.J. Adams, E.H.C. Grant, C.A. Pearl, J.B. Bettaso, W.J. Barichivich, W.H. Lowe, K. True, J.L. Ware, and P.S. Corn. 2010. Low prevalence of chytrid fungus (*Batrachochytrium dendrobatidis*) in amphibians of U.S. headwater streams. Journal of Herpetology 44:253–260.
- Huang, R., and L.A. Wilson. 2013. *Batrachochytrium dendrobatidis* in amphibians of the Piedmont and Blue Ridge Provinces in Northern Georgia, USA. Herpetological Review 44:95–98.
- Hyatt, A.D., D.G. Boyle, V. Olsen, D.B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, et al. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 73:175–192.
- Keitzer, S.C., T.K. Pauley, and C.L. Burcher. 2013. Stream characteristics associated with site occupancy by the Eastern Hellbender, *Cryptobranchus alleganiensis alleganiensis*, in southern West Virginia. Northeastern Naturalist 20:666–677.
- Keitzer, S.C., R. Goforth, A.P. Pessier, and A.J. Johnson. 2011. Survey for the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* in

- Journal of Wildlife Diseases 47:455–458.
- Kiemnec-Tyburczy, K.M., S.L. Eddy, A.J. Chouinard, and L.D. Houck. 2012. Low prevalence of Batrachocytrium dendrobatidis in two plethodontid salamanders from North Carolina. USA. Herpetological Review 43:85–87.
- Kinney, V.C., J.L. Heemeyer, A.P. Pessier, and M.J. Lannoo. 2011. Seasonal pattern of Batrachochytrium dendrobatidis infection and mortality in Lithobates areolatus: affirmation of Vrendenburg's "10,000 Zoospore Rule". **PLoS** ONE 6:e16708. doi:10.1371/journal.pone.0016708
- Kriger, K.M., and J.M. Hero. 2007. Large-scale seasonal variation in the prevalence and severity of chytridiomycosis. Journal of Zoology 271:352–359.
- Lannoo, M.J., C. Petersen, R.E. Lovich, P. Nanjappa, C. Phillips, J.C. Mitchell, and I. Macallister. 2011. Do frogs get their kicks on Route 66? Continental U.S. transect reveals spatial and temporal patterns of Batrachochytrium dendrobatidis infection. PLoS ONE 6:e22211. doi:10.1371/journal.pone.0022211
- Mayasich, J.D., D. Grandmaison, and C. Phillips. 2003. Eastern Hellbender Status Assessment. NRRI/TR-2003/09. U.S. Fish and Wildlife Service, Fort Piotrowski, J.S., S.L. Annis, and J.E. Longcore. 2004. Snelling, Minnesota, USA.
- Meyer, E.A., R.L. Cramp, M.H. Bernal, and C.E. Franklin. 2012. Changes in cutaneous microbial abundance with sloughing: possible implications for infection and disease in amphibians. Diseases of Aquatic Organisms 101:235-242.
- Miller, B.T., and J.L Miller. 2005. Prevalence of physical abnormalities in Eastern Hellbender (Cryptobranchus alleganiensis alleganiensis) populations of middle Tennessee. Southeastern Naturalist 4:513–520.
- Moffitt, D.J. 2012. Occupancy modeling and disease susceptibility of montane salamander species in Western North Carolina, M.Sc. Thesis, Appalachian State University, Boone, North Carolina, USA. 107p.
- Nickerson, C.A., C.M. Ott, S.L. Castro, V.M. Garcia, T.C. Molina, J.T. Briggler, A.L. Pitt, J.J. Tavano, J.K. Byram, J. Barrila, et al. 2011. Evaluation of microorganisms cultured from injured and repressed tissue regeneration sites in endangered giant aquatic Ozark Hellbender salamanders. PLoS ONE 6:e28906. doi:10.1371/journal.pone.0028906
- Nickerson, M.A., and J.T. Briggler. 2007. Harvesting as a factor in population decline of a long-lived salamander; the Ozark Hellbender, Cryptobranchus alleganiensis bishopi. Applied Herpetology 4:207-
- Nickerson, M.A., and K.L. Krysko. 2003. Surveying for salamanders, Cryptobranchus Hellbender alleganiensis (Daudin): a review and critique. Applied Herpetology 1:37-44.

- Southwestern North Carolina salamander populations. Nickerson, M.A., and C.E. Mays. 1973. The Hellbenders: North American Giant Salamanders. Milwaukee Public Museum Publications in Biology and Geology. Milwaukee, Wisconsin, USA.
  - North Carolina Wildlife Resources Commission. 2005. North Carolina Wildlife Action Plan. Raleigh, North Carolina, USA. 577p.
  - Olson, Z.H., N.G. Burgmeier, P.A. Zollner, and R.N. Williams. 2013. Survival estimates for adult Eastern Hellbenders and their utility for conservation. Journal of Herpetology 47:71–74.
  - Peterson, C.L., R.F. Wilkinson, Jr., M.S. Topping, and D.E. Metter. 1983. Age and growth of the Ozark Hellbender (Cryptobranchus alleganiensis bishopi). Copeia 1983:225-231.
  - Pfingsten, R.A. 1990. The status and distribution of the Hellbender, Cryptobranchus alleganiensis, in Ohio. Herpetological Review 21:48-51.
  - Phillips, C.A., and W.J. Humphries. 2005. Cryptobranchus alleganiensis (Daudin, 1803), Hellbender. Pp. 648-651 In Amphibian Declines: The Conservation Status of United States Species. Lannoo, M. (Ed.). University of California Press, Berkeley, California, USA.
  - Physiology of Batrachochytrium dendrobatidis, a chytrid pathogen of amphibians. Mycologia 96:9–15.
  - Pugh, M.W., J.D. Groves, L.A. Williams, and M.M. Gangloff. 2013. A previously undocumented locality of Eastern Hellbenders (Cryptobranchus alleganiensis alleganiensis) in the Elk River, Carter County, TN. Southeastern Naturalist 12:137-142.
  - Pullen, K.D., A.M. Best, and J.L. Ware. 2010. Amphibian pathogen Batrachochytrium dendrobatidis prevalence is correlated with season and not urbanization in central Virginia. Diseases of Aquatic Organisms 91:9-16.
  - Quinn, S.A., J.P. Gibbs, M.H. Hall, and P.J. Petokas. 2013. Multiscale factors influencing distribution of the Eastern Hellbender salamander (Cryptobranchus alleganiensis alleganiensis) in the northern segment of its range. Journal of Herpetology 47:78-84.
  - Regester, K.J., H. Simpson, E.J. Chapman, and P.J. Petokas. 2012. Occurrence of the fungal pathogen Batrachochytrium dendrobatidis among Eastern (Cryptobranchus Hellbender populations alleganiensis) within the Alleghany-Ohio Susquehanna River drainages, Pennsylvania, USA. Herpetological Review 43:90-93.
  - Rizkalla, C.E. 2010. Increasing detections of Batrachochytrium dendrobatidis in Central Florida, USA. Herpetological Review 41:180–181.
  - Rothermel, B.B, E.R. Travis, D.L. Miller, R.L. Hill, J.L. McGuire, and M.J. Yabsley. 2013. High occupancy of stream salamanders despite high Ranavirus prevalence

- in a Southern Appalachians watershed. EcoHealth 10:184–189.
- Rothermel, B.B., S.C. Walls, J.C. Mitchell, C.K. Dodd, Jr., L.K. Irwin, D.E. Green, V.M. Vazquez, J.W. Petranka, and D.J. Stevenson. 2008. Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the Southeastern USA. Diseases of Aquatic Organisms 82:3–18.
- Skerratt, L.F., D. Mendez, K.R. McDonald, S. Garland, J. Livingstone, L. Berger, and R. Speare. 2011. Validation of diagnostic tests in wildlife: the case of chytridiomycosis in wild amphibians. Journal of Herpetology 45:444–450.
- Solis, M.E., J.M. Bandeff, and Y. Huang. 2007. Hematology and serum chemistry of Ozark and Eastern Hellbenders (*Cryptobranchus alleganiensis*). Herpetologica 63:285–292.
- Souza, M.J., M.J. Gray, P. Colclough, and D.L. Miller. 2012. Prevalence of infection by *Batrachochytrium dendrobatidis* and *Ranavirus* in Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in Eastern Tennessee. Journal of Wildlife Diseases 48:560–566.
- Taber, C.A., R.F. Wilkinson, Jr., and M.S. Topping. 1975. Age and growth of Hellbenders in the Niangua River, Missouri. Copeia 1975:633–639.
- Terrell, V.C., N.J. Engbrecht, A.P. Pessier, and M.J. Lannoo. 2014. Drought reduces chytrid fungus (*Batrachochytrium dendrobatidis*) infection intensity and mortality but not prevalence in adult Crawfish Frogs (*Lithobates areolatus*). Journal of Wildlife Diseases 50:56–62.
- Timpe, E.K., S.P. Graham, R.W. Gagliardo, R.L. Hill, and M.G. Levy. 2008. Occurrence of the fungal pathogen *Batrachochytrium dendrobatidis* in Georgia's amphibian populations. Herpetological Review 39:447–449.
- Tominaga A, K.J. Irwin, M.J. Freake, K. Suzuki, and K. Goka. 2013. *Batrachochytrium dendrobatidis* haplotypes on the Hellbender *Cryptobranchus*

- *alleganiensis* are identical to global strains. Diseases of Aquatic Organisms 102:181–186.
- Trauth, S.E., J.D. Wilhide, and P. Daniel. 1992. Status of the Ozark Hellbender, *Cryptobranchus bishopi* (Urodela: Cryptobranchidae), in the Spring River, Fulton County, Arkansas. Proceedings of the Arkansas Academy of Science 46:83–86.
- Unger, S.D., A. Mathis, and R. Wilkinson. 2013a. A comparison of sperm health in declining and stable populations of Hellbenders (*Cryptobranchus alleganiensis alleganiensis* and *C. a. bishopi*). American Midland Naturalist 170:382–392.
- Unger, S.D., T.M. Sutton, and R.N. Williams. 2013b. Projected population persistence of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) using a stage-structured life-history model and population viability analysis. Journal for Nature Conservation 21:423–432.
- Vazquez, V., B. Rothermel, and A. Pessier. 2009. Experimental infection of North American plethodontid salamanders with the fungus *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms 84:1–7.
- Vrendenburg, V.T., R.A. Knapp, T.S. Tunstall, and C.J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Science 107:9689–9694.
- Wheeler, B.A., M.L. McCallum, and S.E. Trauth. 2002. Abnormalities in the Ozark Hellbender, *Cryptobranchus alleganiensis bishopi*. Journal of the Arkansas Academy of Science 56:250–252.
- Wheeler, B.A., E. Prosen, A. Mathis, and R.F. Wilkinson. 2003. Population declines of a long-lived salamander: a 20+-year study of Hellbenders, *Cryptobranchus alleganiensis*. Biological Conservation 109:151–156.
- Williams, R.D., J.E. Gates, C.H. Hocutt, and G.J. Taylor. 1981. The Hellbender: a nongame species in need of management. Wildlife Society Bulletin 9:94–100.

#### Williams and Groves.—Batrachochytrium dendrobatidis in Eastern Hellbenders.



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JOHN D. GROVES has been in the zoo field for 48 y. He began his career at the Baltimore Zoo where he worked as a Keeper and Senior Keeper of Reptiles for seven years. He then went to the Philadelphia Zoological Park as Head Keeper of Reptiles in 1973, and then as Assistant Curator and Curator of Reptiles. He added Curator of Birds at the Philadelphia Zoo to his list of titles in 1986. John continued in these positions until 1993 when he took the position as Curator of Amphibians and Reptiles at the North Carolina Zoological Park. He has recently retired and is currently Curator Emeritus at the North Carolina Zoological Park. John has led and/or participated in research and conservation trips to Bolivia, Peru, Venezuela, Costa Rica, Mexico, Hawaii, and the Mariana Islands. John has also done much research in various parts of the United States. He has been a Research Associate at the Academy of Natural Sciences of Philadelphia (Vertebrate Zoology), a Research Associate (Ornithology) at the North Carolina Museum of Natural Sciences in Raleigh, and a Conservation Associate at the Philadelphia Zoological Garden. (Photographed by Lori Williams).

**APPENDIX A.** Field notes of physical condition (e.g., injuries, anomalies, scarring, etc.) and results of *Batrachochytrium dendrobatidis* (*Bd*) testing of wild-caught Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) by age class, sex, and HUC 6 river basin in Western North Carolina, 2008–2012; one animal where body condition was not recorded is not included. A = Adult; J = Juvenile; F = Female; M = Male; U = Undetermined Sex; FB = French Broad; UpTN = Upper Tennessee; MidTn = Middle Tennessee; Hiw = Hiwassee; LF = Left Front Foot; LR = Left Rear Foot; RR = Right Rear Foot; RF = Right Front Foot.

Hellbender Sample	Age Class	Sex	HUC 6 River Basin	Bd Result	Physical Condition (injuries or anomalies)
2008_01	A	F	FB-Holston	negative	none
2008_02	Α	F	FB-Holston	positive	none
2008 03	A	M	UpTN	negative	none
2008 04	A	M	UpTN	negative	missing LF digits1, 3, 4
2008 05	A	F	UpTN	positive	none
2008_06	A	M	UpTN	negative	no toes on rear feet, 3–4 cm of tail gone, open sore on head over left eye
2008 07	A	F	UpTN	positive	none
2008_07	A	F	UpTN	positive	none
				•	
2008_09	A	F	UpTN	negative	none
2008_10	A	M	UpTN	negative	scar on head, bite marks on end of tail
2008_11	J	U	UpTN	negative	none
2008_12	A	M	UpTN	negative	missing RR; LF digit 3 missing
2008_13	A	F	UpTN	negative	missing RR4, RF digit 2
2008_14	A	M	UpTN	negative	none
2008_15	Α	F	UpTN	negative	RR digits 4,5 reduced; tissue missing from tail
2008 16	A	F	UpTN	negative	notch on tail
2008 17	A	F	UpTN	positive	LF digits 1,3,4 missing
2008 18	A	M	UpTN	negative	none
2008 19	A	F	UpTN	negative	tail notches, injury on LF elbow
2008_20	A	M	UpTN	negative	missing RR digits 1,4
2008_21	A	F	FB-Holston	negative	bite mark on top and bottom of head
2008_21	A	F	FB-Holston	positive	injury on top of head, bottom jaw, cloaca,
_				-	right eye infected
2008_23	J	U	FB-Holston	negative	LF missing two toes; tail tip split; RR digits1, 2 fused
2008_24	A	F	FB-Holston	positive	none
2008_25	Α	M	FB-Holston	negative	RR foot has extra digit
2008_26	J	U	FB-Holston	positive	none
2008_27	A	M	FB-Holston	positive	none
2008_28	A	M	FB-Holston	positive	bite on RR leg;
2000 20			FD 11 1		tumor-like mass on tail; toe missing
2008_29	Α	F	FB-Holston	positive	none
2008_30	A	F	FB-Holston	positive	none
2008_31	J	U	FB-Holston	positive	none
2008_32	A	F	FB-Holston	positive	exposed bone/infectious tissue on RR; stunted toe RF
2008 33	J	U	FB-Holston	negative	none
2008 34	J	U	FB-Holston	positive	old scars on head; missing RF
2009 01	A	F	Kanawha	positive	half of LF digit 2 missing; bump on tail
2009 02	A	F	Kanawha	positive	none
2009 03	J	Ù	FB-Holston	negative	cleft pallet
2009_03	A	F	FB-Holston	positive	none
2009_04	J	U	FB-Holston	positive	notch at top of tail
2009_06	J	U	FB-Holston	positive	none
2009_00	J	U	FB-Holston	negative	none
2009_07	A	U	FB-Holston	positive	
2009_08					none
	A	M	FB-Holston	negative	head scar
2009_10	A	M	FB-Holston	negative	none
2009_11	A	F	FB-Holston	positive	none
2009_12	A	M	FB-Holston	positive	none
2009_13	A	M	FB-Holston	negative	old wounds on head
2009_14	A	M	FB-Holston	negative	none
2009_15	Α	M	FB-Holston	negative	none
2009_16	A	F	FB-Holston	negative	notch on LF elbow
2009_17	J	U	FB-Holston	positive	none
2009_18	J	U	FB-Holston	negative	none
2009_19	Α	F	FB-Holston	positive	none
2009_20	A	M	FB-Holston	negative	old bite marks on head, tail;
				<u> </u>	RF, LF, LR all with missing toes

2009 21	A	M	FB-Holston	negative	LR3,4,5 missing/regenerating
2009 22	A	F	FB-Holston	negative	old bite on tail;
2009_22	Α	1	T-D-HOIStOII	negative	
	_			_	missing portions of RF, LF, LR feet, toes
2009_23	J	U	FB-Holston	negative	none
2009 24	A	F	Kanawha	negative	none
2010 01	J	U	FB-Holston	negative	none
_				-	
2010_02	J	U	FB-Holston	negative	none
2010_03	J	U	FB-Holston	negative	none
2010 04	A	F	Kanawha	negative	none
2010 05	A	M	Kanawha	negative	none
_				-	
2010_06	J	U	Kanawha	negative	none
2010_07	Α	F	Kanawha	positive	none
2010 08	A	F	Kanawha	negative	none
2010 09	Α	M	Kanawha	negative	none
_			Kanawha	•	
2010_10	A	M		positive	old bite or cut on LR
2010_11	Α	M	Kanawha	positive	none
2010 12	Α	F	FB-Holston	negative	none
2010 13	A	U	FB-Holston	negative	none
_	A	F	FB-Holston	-	
2010_14				negative	none
2010_15	A	M	FB-Holston	negative	none
2010 16	J	U	FB-Holston	negative	none
2010 17	A	M	FB-Holston	negative	LF has fused toes
2010 18	A	M	FB-Holston	negative	old wound on tail
_				-	
2010_19	A	M	FB-Holston	negative	old notch on tail
2010_20	Α	U	FB-Holston	negative	LF toes1–3 missing;
					notch on bottom of tail
2010 21	A	U	FB-Holston	negative	notch on tail
_				-	
2010_22	A	F	FB-Holston	negative	none
2010_23	Α	M	FB-Holston	negative	none
2010 24	A	F	FB-Holston	negative	none
2010 25	A	F	Kanawha	negative	LF all toes missing; nares inflamed;
2010_23			Tuna wha	negative	wound on R side of neck near gill slit
		_			
2010_26	A	F	FB-Holston	negative	yellow spot on snout
2010 27	Α	M	Kanawha	negative	LR1 missing
2010 28	A	M	Kanawha	negative	none
2010 29	Α	M	Kanawha	negative	LF digit 3 no toe pad
_				-	
2010_30	A	F	UpTN	negative	LR foot has white fungus
2010_31	Α	F	Kanawha	positive	LF digit 1 missing; o
					ld scar on forehead and chin
2010 32	A	F	MidTN-Hiw	negative	notch on tail
_				-	
2010_33	A	M	MidTN-Hiw	positive	none
2010_34	J	U	MidTN-Hiw	negative	none
2010_35	Α	F	FB-Holston	negative	notch on top of tail regenerating
2010 36	A	U	FB-Holston	negative	none
2010_37	A	M	FB-Holston	negative	old scar on forehead; missing RR digit 3
				•	
2010_38	A	M	FB-Holston	negative	old scar on forehead; RF2 stub toe;
					notch on keel of tail
2010 39	A	M	FB-Holston	negative	old scar on forehead; LR digit 3 missing;
_				2	LR digits 1, 2 short and regenerating
2010 40	A	F	FB-Holston	negative	notch on tail tip
_				-	
2010_41	Α	M	FB-Holston	negative	old bite on tail; notch on tail;
					LR toes regenerating;
					dark pigment on center of head
2010 42	Α	M	FB-Holston	negative	RF digit 3 missing;
2010_42	Α	1V1	TD-Hoiston	negative	
					all other digits on RF foot regenerating
2010_43	Α	F	FB-Holston	negative	none
2010_44	Α	M	FB-Holston	negative	RR4 missing
2010 45	A	M	FB-Holston	negative	notch on tail; RF digit 1 missing
				_	
2010_46	A	M	FB-Holston	negative	none
2010_47	Α	M	FB-Holston	negative	none
2010_48	A	M	FB-Holston	negative	bite on tail
2010_49	A	M	FB-Holston	negative	puncture wound on Right hip;
-010-17		171			white fungus on wound and venter
2010 50	•	* *	ED II I		· ·
2010_50	J	U	FB-Holston	negative	none
2010_51	A	F	FB-Holston	positive	none
2010 52	A	M	FB-Holston	negative	laceration on R forearm;
· · · · · -					missing one rear toe
2010 52	Ť	ŢŢ	ED 11-1-4	magati	
2010_53	J	U	FB-Holston	negative	none
2010_54	J	U	FB-Holston	negative	none
2010_55	J	U	FB-Holston	negative	none
_				-	

## Herpetological Conservation and Biology

2011 01	A	F	MidTN-Hiw	positive	old notch on left side
2011_01	A	Ü	MidTN-Hiw	negative	LR toes short and regenerating
2011_02	A	M	MidTN-Hiw	positive	none
	A	M			
2011_04			MidTN-Hiw	negative	none
2011_05	A	F	MidTN-Hiw	negative	scar on LF foot
2011_06	J	U	MidTN-Hiw	negative	none
2011_07	Α	F	MidTN-Hiw	negative	none
2011_08	Α	F	MidTN-Hiw	positive	notches on posterior tail keel
2011_09	A	F	MidTN-Hiw	negative	none
2011_10	A	M	MidTN-Hiw	negative	notch on bottom of tail tip
2011 11	A	M	MidTN-Hiw	negative	none
2011 12	Α	F	MidTN-Hiw	negative	lower chin has abrasion, raw area
2011 13	Α	M	MidTN-Hiw	negative	none
2011_14	A	F	MidTN-Hiw	negative	RF foot all toes missing,
2011_11		•		negati v	starting to regenerate 1, 2;
					LR digit 1 old abscess;
2011 15		Г	M. ITNI II.	,.	four skin tags on ventral side of tail
2011_15	A	F	MidTN-Hiw	negative	none
2011_16	A	F	MidTN-Hiw	negative	none
2011_17	A	M	MidTN-Hiw	negative	LR has a double toe; old injury on tail,
					possibly from fishing line; RF has red,
					swollen footpad
2011 18	Α	F	MidTN-Hiw	negative	skin tag on RR5
2011 19	Α	M	MidTN-Hiw	negative	LR digit 5 regenerating; bloody lower lip
2011 20	Α	M	MidTN-Hiw	negative	RF digit 3 regenerating;
					old bite mark both sides of tail
2011 21	A	F	MidTN-Hiw	negative	none
2011_21	A	M	MidTN-Hiw	equivocal	none
_	A	M	MidTN-Hiw	positive	none
2011_23					
2011_24	A	M	MidTN-Hiw	negative	scar near R eye; divot in tail keel
2011_25	Α	M	UpTN	positive	LF digits 1, 2 regenerating, 3–5 missing;
					RF digits1,2,3 missing; right eye damage;
					old wounds on left side, base of tail, end of tail
2011_26	Α	F	UpTN	positive	none
2011_27	Α	F	UpTN	negative	RR limb missing (trying to regenerate with 1–2 toes); LR digit
					4 regenerating; skin tag on tail tip
2011_28	A	M	UpTN	negative	notches on tail;
_			•	Č	old discoloration on anterior tail keel
2011 29	Α	M	UpTN	negative	none
2011 30	A	F	UpTN	positive	none
2011_31	A	F	UpTN	positive	scrape L side of neck;
2011_51	2.1	•	ОРТІ	positive	RR digits 2,3,4 bitten off
2011 32	A	M	UpTN	positive	divot in anterior tail keel
_		F			
2011_33	A		UpTN	negative	none
2011_34	A	F	UpTN	positive	none
2011_35	A	M	UpTN	negative	RR4, 5 regenerating
2011_36	A	M	UpTN	positive	RR1 missing; LR foot all toes missing and single toe pad
					regenerating
2011_37	Α	F	UpTN	negative	divot on anterior tail keel;
					joints of leg fused with skin tag; two toes regenerating
2011 38	J	U	MidTN-Hiw	negative	none
2011 39	A	F	MidTN-Hiw	negative	none
2011_40	A	F	MidTN-Hiw	negative	none
2011_41	Α	F	FB-Holston	negative	none
2011_42	A	M	FB-Holston	equivocal	old bite marks/scars on RR foot and tail
2011_43	A	F	FB-Holston	positive	old bite mark/scar on top of tail
2011_43	A	F	FB-Holston	negative	none
				_	
2012_02	A	F	FB-Holston	negative	RR1–4 missing, regenerating
2012_03	A	M	FB-Holston	positive	none
2012_04	A	M	FB-Holston	negative	old scar from bite on venter, lower R
2012_05	Α	M	FB-Holston	negative	none
2012_06	Α	M	MidTN-Hiw	positive	ragged tail tip
2012_07	Α	F	FB-Holston	positive	none
2012_08	A	M	FB-Holston	negative	none