



# Translocation does not influence amphibian chytrid fungus prevalence among wild eastern hellbenders *Cryptobranchus alleganiensis*

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**ABSTRACT:** Disease monitoring is an essential step in translocation projects, specifically in amphibians where emerging pathogens such as the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) are linked to population declines. The eastern hellbender *Cryptobranchus alleganiensis* is a large, fully aquatic salamander experiencing precipitous range-wide population declines; however, the role *Bd* plays in these declines is unclear. To augment declining hellbender populations and determine effects of translocation on *Bd* prevalence, we conducted a translocation study of wild adult hellbenders from 2 source streams with abundant hellbender populations to 2 streams with declining populations in east Tennessee, USA. In 2018, we implanted radio transmitters into 30 hellbenders and sampled them periodically for *Bd* until 17 of the 30 hellbenders were translocated in 2019. We attempted to recapture translocated hellbenders approximately every 45 d for 3 mo to determine *Bd* prevalence post-release. We used qPCR to detect *Bd* and quantify zoospore loads on positive samples. Hellbenders had a pre-translocation *Bd* prevalence of 50% (15 of 30), which decreased to 10% (1 of 10) post-translocation. The average zoospore load for positive samples was  $73.63 \pm 30.82$ , and no hellbenders showed signs of chytridiomycosis throughout the study. Although we detected no significant effect of translocation on *Bd* prevalence, we observed a reduction in *Bd* prevalence post-release. Our results indicate that translocation did not lead to an increase in pathogen prevalence in translocated wild adult hellbenders, suggesting that chytrid did not impact the success of short-term translocations of eastern hellbenders in the Blue Ridge ecoregion.

**KEY WORDS:** Hellbender · Translocation · Chytridiomycosis · *Batrachochytrium dendrobatidis* · Amphibian disease

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## 1. INTRODUCTION

In recent decades, there has been an increase in the prevalence and spread of wildlife diseases, and emerging infectious diseases are now recognized as a major cause of population declines in many species (Smith et al. 2009). The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) is an emerged pathogen

that has caused mass die-offs of amphibian populations across the globe (Scheele et al. 2019, 2020, Lambert et al. 2020). It has been called 'the worst infectious disease ever recorded among vertebrates in terms of the number of species impacted, and its propensity to drive them to extinction' (Gascon et al. 2007, p. 59). Recent estimates suggest that *Bd* has caused the decline of 501 species of amphibians and

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contributed to the extinction of 90 species (Scheele et al. 2019). Despite these documented declines, there are species for which *Bd*-associated impacts are unclear, especially cryptic species that are often challenging to study *in situ*. Collection of baseline data on *Bd* prevalence is essential to understand how this pathogen impacts these species and to better inform conservation management decisions with regards to disease mitigation and management.

*Bd*, a species of aquatic chytrid fungus, is the causative agent of chytridiomycosis, a disease that infects keratinized layers of amphibian skin (Longcore et al. 1999). Clinical signs of chytridiomycosis include lethargy, loss of righting reflex, and increased skin sloughing (Berger et al. 1999). Mortality due to chytridiomycosis is dependent on the infection load (i.e. number of zoospores) crossing a lethal threshold, which can vary by species (Carey et al. 2006, Weinstein 2009, Kinney et al. 2011). Infection loads may increase or decrease throughout time, and this variation in infection load is likely species-dependent (Stockwell et al. 2010). Some amphibian species can become infected with *Bd* but show no clinical signs of chytridiomycosis (Reeder et al. 2012). Monitoring of individuals with subclinical infections is necessary to detect increases in infection loads and to identify potential sublethal effects such as weight loss (Parris & Beaudoin 2004) and reduced foraging efficiency (Hanlon et al. 2015). Thus, deciphering standard *Bd* infection load in species is important to detect infection that may further impact already declining populations.

In addition to the threats that pathogens pose, amphibians are also negatively impacted by habitat loss and fragmentation, climate change, over-collection, and invasive species (Lips et al. 2006, Pounds et al. 2006). To ameliorate species declines, translocations (e.g. augmentation, repatriation) of wild and captive-reared amphibians can be used as a conservation strategy (Dodd & Seigel 1991, Griffiths & Pavajeau 2008, Germano & Bishop 2009). Success of these projects is dependent on a suite of factors including habitat selection, prey availability, and animal health. Disease monitoring and risk assessment are also essential components of translocations that can influence overall success rates (Kock et al. 2010, Suarez et al. 2017). For example, *Bd* was likely responsible for declines of native populations in Europe from a re-introduction of Mallorcan midwife toads *Alytes muletensis* that were inadvertently infected with *Bd* (Walker et al. 2008). Best management and conservation practices, based on project constraints such as financial and time limitations,

should be used to limit disease transmission (Cunningham 1996). Ideally, this would include testing individuals for pathogens before and at least once after translocation to detect changes in pathogen prevalence that may lead to population declines (Mathews et al. 2006). Short- and long-term monitoring of animal health during translocations will allow for proper disease mitigation should an outbreak occur. Methods to control chytridiomycosis outbreaks *in situ* during translocations may include altering environmental conditions (e.g. increasing water salinity and/or temperature) or anti-fungal treatments (Scheele et al. 2014).

Translocated individuals are often susceptible to disease from chronic stress associated with capture, handling, and transport (Dickens et al. 2010). Particularly in amphibians, handling and transport have been shown to increase stress hormone levels (Narayan et al. 2011, Narayan & Hero 2011), and elevated levels of stress hormones have been correlated with harmful effects associated with chytridiomycosis (Peterson et al. 2013). Therefore, translocation may increase stress and lead to increased disease susceptibility. Chytridiomycosis has been linked to high rates of mortality during translocations of amphibians (Stockwell et al. 2008, Adams et al. 2014) and therefore can influence project success. It is imperative to determine the effect of translocations on amphibian species in which the potential impacts of *Bd* are unknown (which may in part be due to limited surveillance). Decreasing the amount of time individuals are handled, reducing re-captures, and careful planning of transport mode may lessen harmful impacts of translocation-related stress and disease (Dickens et al. 2010).

Translocations represent one strategy proposed to augment declining populations of the eastern hellbender *Cryptobranchus alleganiensis alleganiensis*. The eastern hellbender is a large (up to 60 cm in length), long-lived (>20 yr), fully aquatic salamander that typically inhabits cooler, well-oxygenated streams with minimal human disturbance (Smith 1907, Hillis & Bellis 1971, Nickerson & Mays 1973). Historically, the geographic range of eastern hellbenders spanned from southwest New York to northern Georgia and Alabama. A separate subspecies, the Ozark hellbender *C. a. bishopi*, was once found historically throughout the White River watershed in Missouri and Arkansas (Williams et al. 1981). However, populations of both subspecies have declined considerably from historical ranges, and many populations are now locally extirpated (Wheeler et al. 2003, Foster et al. 2009, Graham et al. 2011). The pri-

mary causes of these declines include stream degradation, habitat loss, and over-collection (Nickerson & Briggler 2007, Davis & Hopkins 2013, Jachowski et al. 2016, Unger et al. 2017). Translocation efforts to augment or reestablish declining populations of both subspecies have been undertaken using captive-reared and wild-caught individuals (Gates et al. 1985, Bodinof et al. 2012a, Boerner 2014). Short-term success of these projects has often been monitored via radio telemetry to track survival rates and daily movements (Stouffer et al. 1983, Burgmeier et al. 2011a, Bodinof et al. 2012a, Kraus et al. 2017). Translocation coupled with radio-telemetry provides an opportunity to locate and recapture the same individuals repeatedly for continual health monitoring. This is especially valuable for species like hellbenders that can be difficult to locate *in situ* and often remain hidden under large boulders and stream banks.

Pathogen and disease monitoring for the duration of a hellbender translocation study is critical because aquatic pathogens, specifically *Bd*, have been confirmed in many populations (Burgmeier et al. 2011b, Souza et al. 2012, Williams & Groves 2014, Bales et al. 2015). Furthermore, the stress of translocation may lead to increased infection, which could influence survival of hellbenders post-release (Dean et al. 2016, Kocher 2019). Wild hellbender populations typically have a *Bd* prevalence of <25% (Burgmeier et al. 2011b, Eskew et al. 2014, Bales et al. 2015), although populations with greater prevalence (>45%) have been reported (Gonynor et al. 2011, Seeley et al. 2016, Kocher 2019). In addition, individuals that test positive for *Bd* generally have low zoospore loads and show no clinical signs of chytridiomycosis (Williams & Groves 2014, Bales et al. 2015). However, hellbenders that are captive-reared or involved in translocation efforts have greater *Bd* prevalence and susceptibility post-release compared to individuals in wild populations (Utrup & Mitchell 2008, Bodinof et al. 2012b, Boerner 2014, Dean et al. 2016, Kocher 2019). For example, 7 adult, captive-reared hellbenders succumbed to *Bd* infection during a translocation effort in New York State, USA (Dean et al. 2016). In addition, 22 out of 25 animals that were translocated tested positive for *Bd* (Dean et al. 2016). As few studies have evaluated the impact of *Bd* on translocations before and after release, and *Bd* has been shown as a factor that can impact animal survival, it is important to evaluate prevalence and signs of disease before and after release to determine effects of translocation on hellbender health.

The primary goals of this study were to monitor *Bd* prevalence in wild adult hellbenders for 1 yr before and after translocation and to determine factors that potentially influence *Bd* prevalence. Relatively few studies of hellbenders have sampled for *Bd* repeatedly among the same individuals, and none have experimentally evaluated the effects of translocation on *Bd* prevalence. To that end, the objectives of this study were to (1) collect *Bd* samples from 30 eastern hellbenders in source streams 1 yr prior to translocation, immediately prior to translocation, and approximately 45, 90, and 135 d post-translocation, (2) determine *Bd* zoospore load on positive samples, and (3) test effects of site, individual sex, animal size, and translocation status on *Bd* prevalence. We predicted (1) *Bd* prevalence would be below 50% in source populations based on results from similar studies in our study area (Souza et al. 2012, Tominaga et al. 2013), (2) *Bd* zoospore load would be low (<1000 zoospores) on positive hellbenders, as individuals in east Tennessee typically have low zoospore loads (Hardman et al. 2020a), and (3) study site, individual sex, or animal size would not influence *Bd* prevalence, but translocation would lead to greater *Bd* prevalence post-release as shown in previous studies (Dean et al. 2016, Kocher 2019). Collectively, this study provides data on the dynamics of *Bd* before and after translocation and evaluates whether site- or animal-specific factors impact *Bd* prevalence.

## 2. MATERIALS AND METHODS

### 2.1. Site selection

Eastern hellbenders are found in 15 states in the eastern and midwestern USA (USFWS 2018). Within Tennessee, they occur only in streams and rivers with Tennessee and Cumberland River drainages. We selected source populations based on genetics, population size, and ecological importance of the streams they inhabit (i.e. viable, healthy population within a protected area) (Freake & DePerno 2017). We only used populations that consisted of all age classes (i.e. larvae, juveniles, and adults) as source populations. These considerations limited our site selection to streams in the Upper Tennessee River drainage in the Blue Ridge ecoregion that are surrounded by protected areas in the Cherokee National Forest. We do not provide stream names or site locations to protect the populations of this state endangered species from potential poaching. Source Stream 1 (SS1) served as the source population for individuals translocated to

Translocation Stream 1 (TS1), and Source Stream 2 (SS2) served as the source population for individuals translocated to Translocation Stream 2 (TS2). Hellbenders were only translocated between streams within watersheds that share similar genetics based on Freake et al. (2018).

TS1 was chosen because hellbenders historically inhabited this stream, but due to large dams, the remaining population is now isolated from others, including SS1, and population densities are low with no recent recruitment or breeding observed (<5 individuals; M. Freake unpubl. data). Prior to impoundment, similar genetic profiles (Freake et al. 2018) indicate that hellbenders likely moved freely between SS1 and TS1. We selected TS2 because hellbenders were known to inhabit this stream (Mayasich et al. 2003), but recent surveys indicate the population is extirpated or nearly so, as no individuals have been found since 2015 (M. Freake unpubl. data), and recent environmental DNA (eDNA) analysis in 2016 failed to detect hellbender presence (Da Silva Neto et al. 2020). Similar to TS1, declines in this stream also are likely caused by a lack of recruitment and isolation from other hellbender populations due to dams present across the watershed.

## 2.2. Hellbender surveys

We conducted initial hellbender surveys during June and July 2018. All hellbenders were handled with the approval of Tennessee State University's Institutional Animal Care and Use Committee IACUC permit #1804WS and Tennessee Wildlife Resource Agency collecting permit #1685 and #2192. At SS1 and SS2, we surveyed predetermined stream reaches (~100–500 m) for hellbenders by standard mask-and-snorkel rock-lifting surveys. We captured hellbenders with clean gloves and placed individuals inside mesh bags for transport to individual holding tubs at a processing station on shore. We recorded GPS coordinates of the capture location and measured mass (g), snout–vent length (SVL; cm), and total length (TL; cm) for each animal. No hellbenders <150 g were included in further processing.

## 2.3. *Bd* sampling and surgery protocols

To sample for *Bd*, we swabbed each animal with a sterile cotton swab (Puritan 25-806) 5 times back and forth over the dorsum, lateral surface, and each leg and foot. All swabs were immediately placed in pre-steril-

ized dry 1.5 ml tubes and stored on dry ice up to 48 h until transferred to a –80°C freezer. We then collected blood (up to 0.25 ml) via phlebotomy of the ventral tail vein to genetically determine sex as described in Hime et al. (2019). After sampling at the initial capture, we anesthetized hellbenders with MS-222 buffered with baking soda to a pH of 6.8–7.2 in preparation for transmitter implantation surgery. Animals were considered at an appropriate plane of anesthesia when loss of righting reflex was observed. Surgery was performed by R.H. (DVM, PhD) following procedures similar to Burgmeier et al. (2011a), with some modifications. Dilute chlorhexidine solution was used to prepare a 2 cm<sup>2</sup> area of the ventral skin surface and then rinsed immediately with sterile water. A 1.5 cm paramedian incision was made in the caudal coelom, and a series F1170 ATS model transmitter (Advanced Telemetry Systems) was inserted into the coelomic cavity after being soaked in chlorhexidine for 5 min and rinsed thoroughly with sterile water. In addition to the radio transmitter, a passive integrated transponder (PIT) tag (Biomark) was inserted into the coelomic cavity to provide an additional permanent mark to identify individuals. The body wall was closed with 4-0 polydioxanone (PDS) suture in a simple continuous pattern, and the skin was closed with 5–7 simple interrupted sutures using 4-0 nylon. Following completion of surgery, cef-tazidime (20 mg kg<sup>-1</sup>) was injected intramuscularly at the base of the tail. Hellbenders were placed inside recovery tubs in the stream to allow water to flow freely over individuals until righting reflexes (~30 min) and full activity (~45–60 min) were observed. We returned individuals to their original capture location after complete recovery. We sanitized holding tubs and equipment with 10% bleach and wore new nitrile gloves when handling each hellbender. All animals that were implanted with transmitters were tracked via radio telemetry on a specific tracking schedule for 1 yr until translocations occurred. During this period, we opportunistically recaptured hellbenders to sample for *Bd* and recorded biometrics if the animal was accessible (under cover objects small enough to lift) and had not exhibited large movements since the time of last location (>50 m) to limit stress associated with frequent recaptures.

## 2.4. Translocations

We located and recaptured hellbenders in source streams beginning May 2019 for translocation. The number of hellbenders translocated from each source stream was dependent on accessibility of individuals

as well as survival from the previous season. We translocated individuals in separate cohorts (A, B, C, and D) in each source stream to monitor initial translocations to ensure a successful release. In Cohort A, 4 animals were moved from SS1 to TS1 on 15 May 2019, and in Cohort B, 6 animals were moved from SS2 to TS2 on 18 May 2019. In Cohort C, one animal was moved from SS1 to TS1 on 1 July 2019, and in Cohort D, 5 animals were moved from SS2 to TS2 on 29 June 2019. One individual that was previously inaccessible was translocated opportunistically from SS2 to TS2 on 13 July 2019. This individual was not included in *Bd* analysis as swab storage was compromised. Prior to translocations, we measured and recorded individual metrics, which included mass, SVL, and TL, and collected *Bd* swab samples as described above. We transported hellbenders to translocation streams in separate sterile 3 mm polyethylene commercial-grade fish bags (20" [51 cm] wide × 30" [76 cm] long, sealed bottom; Pentair PLC), filled with source stream water that were placed inside YETI® coolers with ice packs to maintain a cold temperature. Upon arrival at the translocation streams, we placed the fish bags with hellbenders in the stream to closely monitor for signs of stress (vomiting, rocking motions). If hellbenders exhibited stress, we waited to move or release individuals until these signs stopped. During this acclimation period, 50% water changes using translocation stream water was added to the fish bags every 10 min until temperature, pH, and dissolved oxygen inside the bag matched that of the translocation stream. We released hellbenders under naturally occurring cover objects (TS1 and TS2) or hellbender nest boxes (in TS2 only; Briggler & Ackerson 2012) that were installed 1 mo prior to hellbender translocation. We recorded GPS locations after the individual was sedentary under the cover object for >3 min. All translocated hellbenders were tracked approximately every 3 d during the summer and fall (May–November), which included the hellbender breeding season, until the first hard frost of the season (−2°C) on 11 November 2019. We did not attempt to recapture individuals for additional *Bd* samples after this date as water temperatures and conditions became unsafe to survey.

To test for *Bd* prevalence post-translocation, we attempted to recapture and resample hellbenders at approximately 45, 90, and 135 d post-translocation. The duration of time between capture events was chosen to allow an initial acclimatization period and to avoid frequent disturbances that may cause unnecessary stress or movements. During a resample event, all available hellbenders at a translocation site were recaptured in 1 d. Because we translocated

hellbenders in separate cohorts, those translocated in earlier cohorts had the potential for greater resampling events. For example, the first recapture post-translocation for hellbenders in the second cohort would be the second recapture for those translocated in the first cohort.

## 2.5. Laboratory methods

We extracted DNA from swabs using DNeasy Blood and Tissue kits (Qiagen) following the manufacturer's protocols. All samples and standards were eluted with 100 µl AE buffer for further PCR analysis. We tested samples with real-time quantitative polymerase chain reaction (qPCR) for the presence of *Bd* via previously described probe and primers (Boyle et al. 2004) that followed the methods described in Hardman et al. (2020b). Samples were run in triplicate on a QuantStudio 5 System (Applied Biosystems) and recorded as positive if 2 or more of the 3 triplicates were positive for *Bd*. Singlicate positives were re-run to determine presence or absence of *Bd*. Negative and positive controls were included in each qPCR run. We considered samples positive for *Bd* if they had an average cycle threshold ( $C_T$ ) value <40. To calculate zoospore loads for each sample, a standard curve was created based on 10-fold serial dilutions of DNA extracted from cultured zoospores from  $10^6$  to  $10^0$  zoospores.

## 2.6. Statistical analysis

### 2.6.1. *Bd* prevalence

We calculated *Bd* prevalence with 95% CIs at discrete time points throughout the study. First, we calculated *Bd* prevalence at time of initial capture for all individuals (2018,  $n = 30$ ). Secondly, we calculated prevalence from all positive samples from repeated captures of individuals from 2018–2019 ( $n = 30$ ), and then only from translocated individuals (2019,  $n = 16$ ). We calculated total post-translocation *Bd* prevalence in resampled individuals (2019,  $n = 10$ ), as well as for each resample event (#1,  $n = 10$ ; #2,  $n = 5$ ; #3,  $n = 2$ ). In addition, we determined total prevalence by site.

### 2.6.2. Factors influencing *Bd*

We allocated sample data into 3 subsets to evaluate the following: (1) factors influencing *Bd* status pre-

translocation (2018–2019,  $n = 49$ ), (2) factors influencing *Bd* status post-translocation (2019,  $n = 17$ ), and (3) effect of translocation on *Bd* status (2019,  $n = 53$ ). We first performed a generalized linear model (binomial response) in R Studio v.4.0.0 (RStudio Team 2020) to test the effect of sampling year on *Bd* status (positive or negative) for all pre-translocation samples from 2018–2019 to justify combining repeated samples from individuals into one pre-translocation data set. We detected no effect of year on *Bd* prevalence ( $\beta = -0.33 \pm 0.67$ ; 95% CI =  $-1.65, 1.0$ ), therefore, we combined the 2018–2019 pre-translocation data into a single data set.

We tested effects of stream, individual sex, and animal size (SVL) on *Bd* status both pre- and post-translocation separately. To do this, we used the 'lme4' package (Bates et al. 2012) to fit generalized linear mixed models (GLMM) with a binomial distribution and included individual hellbender as a random effect to control for repeated sampling of the same individual. We developed 10 models for each analysis (pre- and post-translocation) using an all subsets approach that included a null and global model, along with nested and additive models. We ranked models using Akaike's information criterion adjusted for small sample size (AICc) via the 'AICcmodavg' package (Mazerolle 2019). For models with  $\Delta AICc < 2.0$ , we calculated model-averaged beta coefficients for variables that were included in more than one model along with standard errors and 95% CIs. To test the effect of translocation on *Bd*, we used a GLMM with translocation as

a fixed effect and *Bd* status as the response variable, while controlling for repeated samples within individuals (random effect).

### 3. RESULTS

#### 3.1. Sampling

We sampled 30 adult hellbenders *Cryptobranchus alleganiensis* (14 females and 15 males; mean mass and SVL: 281.17 g and 23.78 cm, respectively) (SS1,  $n = 13$ ; SS2,  $n = 17$ ) and collected 71 *Bd* samples from 3 June 2018 to 2 October 2019 (Table 1). Of the 30 hellbenders, one individual was sampled 6 times; 6 different individuals were sampled 4, 3, and 2 times, respectively; and 11 individuals were sampled once (Fig. 1).

We resampled all 4 cohorts (A–D) post-translocation at select time intervals based on weather, team availability, and hellbender movement and accessibility. At TS1 for Cohort A, we resampled 3 of 4, 2 of 4, and 1 of 4 individuals 46, 92, and 139 d post-translocation, respectively, and 1 of 1 individual from Cohort C, 45

Table 1. Number of eastern hellbenders *Cryptobranchus alleganiensis alleganiensis* sampled at 2 pre- and 2 post-translocation sites in 4 streams in east Tennessee, USA, and number of *Batrachochytrium dendrobatidis* (*Bd*) samples collected from these hellbenders

Sample date	Site	Hellbenders	Males	Females	Samples	Total
Pre-translocation (2018–2019)	SS1	13	8	5	24	54
	SS2	17	8	9	30	
Post-translocation (2019)	TS1	5	4	1	8	17
	TS2	12	6	6	9	

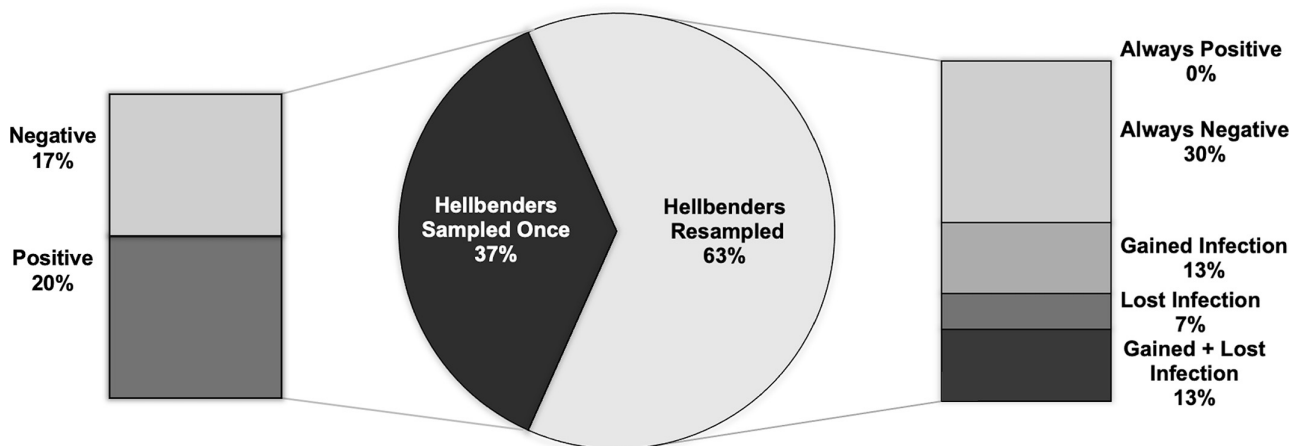


Fig. 1. *Batrachochytrium dendrobatidis* (*Bd*) status (positive or negative) for hellbenders that were sampled only once and for those that were sampled more than once (always positive, always negative, gained *Bd* infection, lost *Bd* infection, and gained and lost *Bd* infection)

and 90 d post-translocation (Fig. 2). At TS2 for Cohort B, we resampled 5 of 6 and 2 of 6 individuals 41 and 90 d post-translocation, respectively, and 1 of 5 individuals from Cohort D 48 and 94 d post-translocation (Fig. 2). For TS2, we observed heavy mortalities due to otter predation between the second and third resampling events. This predation resulted in little to no third *Bd* resampling of those cohorts.

### 3.2. *Bd* prevalence

#### 3.2.1. Pre-translocation

Total *Bd* prevalence in hellbenders between both source streams at initial capture in 2018 was 27% (8 of 30; 95% CI = 12–46%). SS1 had an initial prevalence of 31% (4 of 13; 95% CI = 9–61%), whereas SS2 had an initial prevalence of 24% (4 of 17; 95% CI = 7–50%). Total pre-translocation *Bd* prevalence from all hellbenders sampled was 50% (15 of 30; 95% CI = 31–69%) (Table 2). Immediately prior to translocation in 2019, we resampled 16 of 17 translocated hellbenders between the 2 source streams, of which 25% (4 of 16; 95% CI = 7–52%) were *Bd*-positive (Table 3). SS1 (Cohorts A and C) had pre-translocation prevalence of 40% (2 of 5; 95% CI = 5–85%), and SS2 (Cohorts B and D) had pre-translocation prevalence of 18% (2 of 11; 95% CI = 2–52%).

#### 3.2.2. Post-translocation

At TS1, 4 of 5 hellbenders resampled from both cohorts A and C were negative for *Bd* (0 of 4, 0%) post-translocation (Table 3). At TS2, 1 of 6 individuals tested positive for *Bd* 41 d post-translocation

Table 3. Sample event and total number of individual hellbenders sampled at each site; *Batrachochytrium dendrobatidis* (*Bd*) prevalence is presented with 95% CIs

Sample event	Site (cohort/s)	Hellbenders sampled	<i>Bd</i> prevalence
Pre-translocation (2019)	SS1 (A, C)	5	40 (5–85)
	SS2 (B, D)	11	18 (2–52)
Post-translocation #1 (2019)	TS1 (A, C)	4	0
	TS2 (B, D)	6	17 (0–64)
Post-translocation #2 (2019)	TS1 (A, C)	3	0
	TS2 (B, D)	3	0
Post-translocation #3 (2019)	TS1 (A)	1	0
	TS2 (B, D)	0	0

Table 2. Sampling period and total number of individual hellbenders sampled at each site; *Batrachochytrium dendrobatidis* (*Bd*) prevalence is presented with 95% CIs

Sample time	Site	Hellbenders sampled	<i>Bd</i> prevalence	Total <i>Bd</i> prevalence
Pre-translocation (2018–2019)	SS1	13	69 (39–90)	50 (31–69)
	SS2	17	35 (14–61)	
Post-translocation (2019)	TS1	4	0	10 (0–45)
	TS2	6	17 (0–64)	

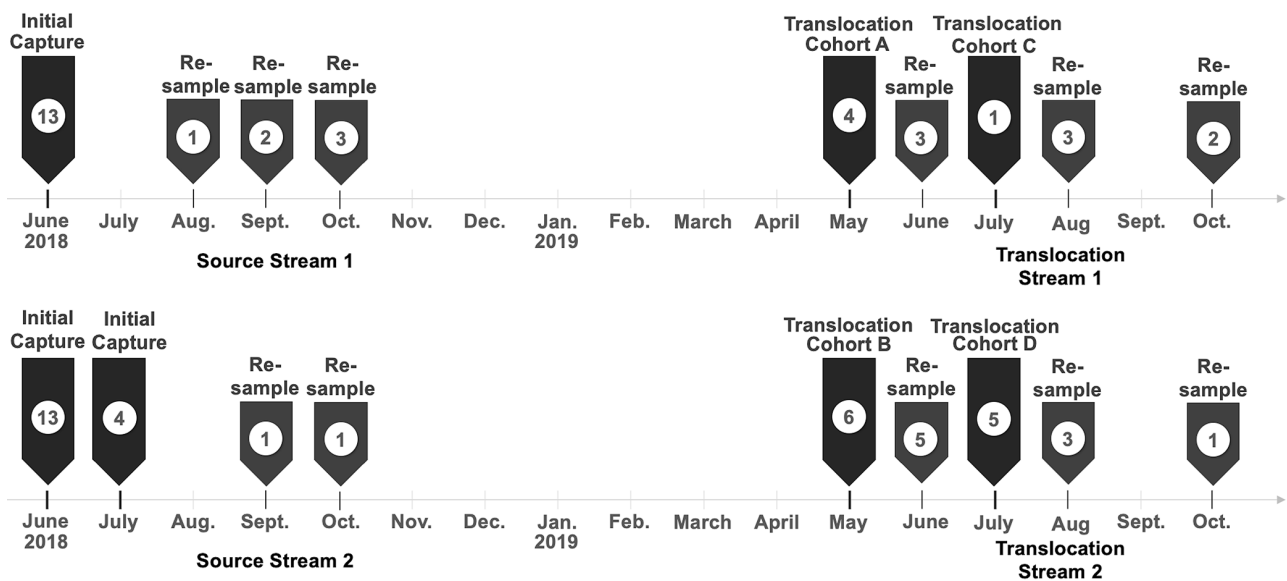


Fig. 2. Timeline depicting duration of study and months when hellbender sampling and translocation occurred. Numbers inside circles: total individuals that were sampled or resampled at that time point

(17%, 95% CI = 0–64%). Total post-translocation *Bd* prevalence for resampled hellbenders was 1 of 10 (10%, 95% CI = 0–45%) (Table 2).

### 3.2.3. Zoospore loads

Average zoospore load for all *Bd*-positive samples ( $n = 17$ ) throughout the study was  $73.63 \pm 30.82$ . However, after removing 3 outliers, average zoospore load dropped to  $24.32 \pm 4.74$  ( $n = 14$ ). Most positive samples (82%) had <100 zoospores. The maximum zoospore load recorded was 499.52, whereas the minimum was 9.83 (Fig. 3). No hellbenders showed behavioral or physical signs of chytridiomycosis at any point during the study.

### 3.2.4. Factors influencing *Bd*

We did not detect an effect of year on *Bd* occurrence pre-translocation ( $\beta = -0.33 \pm 0.67$ ; 95% CI = -1.65, 1.0); therefore, we included all repeat data from individuals in further analyses. For the pre-translocation data set, we found greatest support ( $\Delta\text{AICc} < 2.0$ ) for the following models: (1) stream + SVL, (2) SVL only, and (3) stream only (Table 4). Specifically, source stream ( $\beta = 1.12 \pm 0.64$ ; 95% CI = -0.14, 2.37), and SVL ( $\beta = 0.03 \pm 0.13$ ; 95% CI = -0.22, 0.28) were found in all top-performing models but had CIs that overlapped zero. For the post-translocation data set, we found no significant effect of any measured parameters on *Bd* and found greatest support for the null model (Table 4). We did not detect an effect of translocation on *Bd* prevalence ( $\beta = 1.35 \pm 1.11$ ; 95% CI = -0.83, 3.53).

## 4. DISCUSSION

Translocation of wild adult eastern hellbenders did not lead to an increase in *Bd* prevalence post-release in the Blue Ridge Ecoregion. Despite a smaller sample size post-translocation, we noted a decrease in *Bd* prevalence following release. Although emerging pathogens, specifically *Bd*, have been hypothesized to be potential drivers of hellbender declines (Brig-

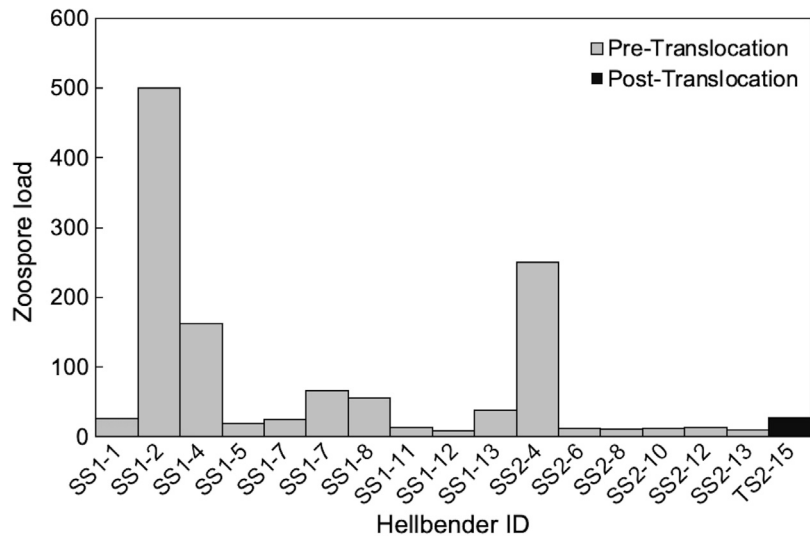


Fig. 3. Zoospore loads for *Batrachochytrium dendrobatidis* (*Bd*)-positive hellbenders pre- and post-translocation

Table 4. Top supported models ( $\Delta\text{AICc} < 2.0$ ) ranked by Akaike's information criterion for small sample size (AICc) for generalized mixed linear models (GLMM) to test effects of predictor variables (stream, individual sex, and animal size [snout–vent length, SVL]) on *Batrachochytrium dendrobatidis* (*Bd*) status (0/1, response variable) using individual hellbender as a random effect

Data set	Model	$K$	AICc	$\Delta\text{AICc}$	AICcWt	Cum. Wt
Pre-translocation ( $n = 54$ )	Stream + SVL	4	67.37	0.00	0.32	0.32
	SVL	3	68.27	0.90	0.21	0.53
	Stream	3	69.11	1.74	0.14	0.66
Post-translocation ( $n = 17$ )	Null	2	8.02	0.00	0.55	0.55

ler et al. 2008, Bodinof et al. 2012b), we did not observe any hellbenders exhibiting signs of chytridiomycosis and did not record deaths associated with chytrid pre- or post-translocation. Prevalence of *Bd* in hellbender populations varies from 0.88% (Eskew et al. 2014) to 52% (Seeley et al. 2016) across the range of the species. In east Tennessee, *Bd* prevalence is intermediate compared to other regions (28–36%; Tominaga et al. 2013, Hardman et al. 2020a). Populations in our streams consistently exhibited low *Bd* prevalence at any given time; however, more than half (16 of 30, 53%) of the individuals tested positive at some point.

Although most hellbenders (53%) tested positive for *Bd* throughout our study, infection intensities were consistently low, with 82% of samples having <100 zoospores. Including outliers, average zoospore load for *Bd*-positive hellbenders was  $73.63 \pm 30.82$ , which is well below the predicted threshold for population die-offs in anurans (10 000 zoospores; Vre-



denburg et al. 2010). Other studies that report zoospore loads in wild hellbenders have found similar results, with most samples having <100 zoospores (Williams & Groves 2014, Bales et al. 2015). The maximum zoospore load recorded in this study was 499.52, which is one of the higher reports for a wild hellbender (Hardman et al. 2020a).

The prevalence of *Bd* in hellbender populations tends to be high in early summer months (Gonynor et al. 2011, Bales et al. 2015) and at high elevations (Seeley et al. 2016). The decrease in *Bd* prevalence we noted after translocation is possibly an artifact associated with our sampling during the warmer months of the year (June–October). Growth of zoospores is inhibited at 28°C (Piotrowski et al. 2004), and the water temperature of the translocation streams reached 27.5°C post-release. Translocation of hellbenders while water temperatures are high may be beneficial to ameliorate the effects of *Bd* and possibly prevent infection and disease. Relatively cooler water temperatures associated with pre-translocation conditions may have accounted for elevated *Bd* prevalence. Most hellbender surveys and translocations occur from May–September when water temperatures are optimal for sampling (Bodinof et al. 2012b, Kraus et al. 2017, Kocher 2019). Little data exists on pathogen prevalence in wild populations outside of this sampling period; consequently, seasonality of *Bd* in wild hellbenders throughout the year is unknown. Translocation studies that sample hellbenders for *Bd* at least once per month could provide valuable year-round pathogen prevalence data; however, sampling should be restricted to periods that do not interfere with important behaviors such as breeding and nesting.

Repeated-sample pathogen data on individual hellbenders is rare, and in our study we collected samples from the same individuals over a 2 yr period. We observed *Bd*-positive hellbenders clear infection and *Bd*-negative hellbenders gain infection. Hellbenders were also able to persist with low-level (<100 zoospores) *Bd* infections and showed no signs of chytridiomycosis. Hellbender disease susceptibility is strongly linked to the immune functions of amphibian skin, including the presence of *Bd*-inhibiting bacteria and production of antimicrobial peptides (Rollins-Smith et al. 2002, Harris et al. 2009). These skin-associated bacterial communities can vary among and between species and can account for differences in disease susceptibility (McKenzie et al. 2012). Arrick (2018) demonstrated that anti-fungal bacteria are present on hellbender skin and may represent a core component of these

skin-associated microbial communities. Immune defense of hellbender skin may be contributing to these low-level *Bd* infections present in hellbender populations. Further exploration is necessary to understand the role of skin microbial communities and *Bd* prevalence.

Long-term pathogen monitoring data is beneficial because it can provide important information on disease dynamics within populations and individuals. Continued intra-individual testing for one or more skin health parameters (e.g. skin microbial communities) alongside *Bd* infection status may provide insight into the potential impacts of *Bd* on hellbender populations. Although collection of repeat-sample data is important, caution should be taken to avoid frequent disturbances that may induce stress or cause animals to move out of the study area. Especially in translocations, individuals should not be disturbed if there is evidence of potential breeding. In our study, we tracked and located 2 hellbenders under the same cover object during the breeding season and did not attempt to resample these individuals. Choice of an appropriate amount of time between sampling events and being mindful of frequency of resampling should be taken into account in these studies.

No hellbenders showed clinical signs of chytridiomycosis at the time of translocation; however, because of our protocol we inadvertently translocated some hellbenders that were *Bd*-positive. *Bd* is widespread in streams throughout the Southeast and Tennessee (Rothermel et al. 2008, Souza et al. 2012), and hellbenders and other fully aquatic amphibians that have tested positive for *Bd* have not experienced mass die-offs (Chatfield et al. 2012, Hardman et al. 2020a). Furthermore, preliminary results from eDNA water samples collected from translocation streams in this study revealed that *Bd* was present at both sites (authors' unpubl. data). Therefore, it is unlikely that we introduced *Bd* into naive environments or that our inadvertent release of hellbenders with *Bd* will contribute to chytrid outbreaks in the translocation streams.

The absence of chytridiomycosis symptoms in wild populations in east Tennessee is consistent with studies of wild populations in other regions (Gonynor et al. 2011, Regester et al. 2012, Williams & Groves 2014). However, a possible association exists between *Bd* prevalence and the occurrence and severity of toe lesions in the closely related Ozark hellbender *C. a. bishopi* (Hardman et al. 2020b). Hellbenders can gain and lose infection over time, with no apparent signs of chytridiomycosis (our study), indicating

that wild eastern hellbenders likely maintain sub-clinical *Bd* infections with minimal health effects. This pathogen is widespread in hellbender populations, but the association of *Bd* to declines in populations is unclear. More long-term studies are needed to elucidate effects of *Bd* infection in wild hellbender populations.

When sustainable source populations are available, the inclusion of wild adults may be advantageous over captive-reared juveniles or adults for translocation projects. Unlike wild amphibians that are exposed to pathogens in their environment throughout development, head-started and captive-reared individuals may be raised in sterile settings free from natural substrate and pathogens. This may lead to decreased immune function and increased disease susceptibility in captive individuals exposed to pathogens (Kueneman et al. 2016). Captive-reared hellbenders have greater *Bd* prevalence post-release (Bodinof et al. 2012b, Dean et al. 2016, Kocher 2019) compared to the wild individuals evaluated during the current study. For example, in a study by Kocher (2019), 20 adult captive-reared hellbenders were released to a stream in New York, USA, and all hellbenders tested positive for *Bd* post-release within approximately 4 mo, with 4 succumbing to chytridiomycosis. In addition, hellbenders that were *Bd*-positive had zoospore loads considerably greater (>5000 intergenic spacer [ITS]-1 copies) than those observed in our study (Kocher 2019). In a separate study, 100% of captive juvenile hellbenders tested negative for *Bd* pre-release; however, after release, 16% (4 of 24) tested positive for *Bd* and one individual died from chytridiomycosis (Bodinof et al. 2012b). These results, combined with our study, suggest that using wild adult hellbenders for translocation efforts may come with low risk of *Bd* infection and should be considered as an alternative to captive-reared juveniles if healthy, wild source populations exist. However, this may be a challenge as many hellbender populations are declining and the number of streams with viable populations are uncommon throughout the range. We recommend researchers and wildlife managers conduct extensive surveys, use current and historical population data, and employ knowledge from experts in the area before selection of wild hellbender source population for a given translocation effort.

As translocations and captive releases become a more common wildlife conservation and management strategy for hellbenders and other species, we suggest the inclusion of pathogen screening and monitoring as an essential step to ensure success of these conservation efforts. Conducting a disease risk

analysis will help determine potential modes of pathogen transmission and risk of species infection during these translocations to mitigate disease outbreaks (Pessier & Mendelson 2010, Suarez et al. 2017). For species like the hellbender in which associations between disease and population declines remain unclear, collection of baseline data prior to translocation is important to understand normal pathogen levels. Pathogen surveillance for the duration of a translocation will allow researchers to detect fluctuations in prevalence so that action can be taken to mitigate disease risk.

Wild adult hellbenders may be more suitable for translocations than captive individuals because wild individuals are likely better adapted to natural disease dynamics in their environment. However, this may be a difficult task as wild hellbender populations continue to decline, and few viable source populations remain. If feasible, future studies should use a combination of wild and captive-reared hellbenders to evaluate differences in pathogen and disease prevalence to determine best practices for translocation. We demonstrated that 1 yr post-translocation, wild adult eastern hellbenders in the Blue Ridge ecoregion are able to persist with low-level *Bd* infections and can be successfully used in translocations without development of chytridiomycosis.

*Acknowledgements.* The authors thank the US Fish and Wildlife Service Competitive State Wildlife Grant Program (Fund # SE-U2-17AP00752), Tennessee State University, Tennessee Wildlife Resources Agency (TWRA), and the Tennessee Herpetological Society Chadwick Lewis Memorial Grant for funding. We also thank the TWRA for providing scientific permits to survey for and translocate wild hellbenders and M. Grove for access to streams in the Cherokee National Forest. We thank B. Buteau, N. Witzel, J. G. Da Silva Neto, S. Snyder, L. Horton, M. LeSage, R. Jackson, and K. McGeary for assistance with hellbender surveys; and S. Doro Reinsch, E. Hall LeSage, J. McKeever, and E. Thornton for assistance with transmitter implantation surgeries. We thank Lincoln Memorial University for assistance with installation of nest boxes at TS2. We thank Animal Medical Center (Murfreesboro, TN) and the Nashville Zoo at Grassmere (Nashville, TN) for hellbender surgery supplies. The authors thank 2 anonymous reviewers for comments on earlier versions of the manuscript.

#### LITERATURE CITED

- ✦ Adams SL, Morton MN, Terry A, Young RP and others (2014) Saving the mountain chicken: long-term recovery strategy for the critically endangered mountain chicken 2014–2034. Mountain Chicken Recovery Programme, Plymouth. <https://www.mountainchicken.org/resources/reports/>
- Arrick RF (2018) Influence of developmental stage, habitat, and captivity on the cutaneous bacterial communities of

- eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in West Virginia. MS thesis, Marshall University, Huntington, WV
- ✦ Bales EK, Hyman OJ, Loudon AH, Harris RN and others (2015) Pathogenic chytrid fungus *Batrachochytrium dendrobatidis*, but not *B. salamandrivorans*, detected on eastern hellbenders. PLOS ONE 10:e0116405
- Bates D, Maechler M, Bolker B, Walker S and others (2012) Package 'lme4'. CRAN. R Foundation for Statistical Computing, Vienna, Austria.
- Berger L, Speare R, Hyatt A (1999) Chytrid fungi and amphibian declines: overview, implications and future directions. In: Campbell A (ed) Declines and disappearances of Australian frogs. Environment Australia, Canberra, p 23–33
- ✦ Bodinof CM, Briggler JT, Junge RE, Beringer J and others (2012a) Postrelease movements of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*). Herpetologica 68:160–173
- ✦ Bodinof CM, Briggler JT, Junge RE, Mong T and others (2012b) Survival and body condition of captive-reared juvenile Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) following translocation to the wild. Copeia 2012:150–159
- Boerner JA (2014) Comparison of movement patterns in captive-released eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) using three different release methods. MA thesis, Buffalo State College, Buffalo, NY
- ✦ 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
- Briggler JT, Ackerson JR (2012) Construction and use of artificial shelters to supplement habitat for hellbenders (*Cryptobranchus alleganiensis*). Herpetol Rev 43:412–416
- Briggler JT, Larson K, Irwin A, Irwin KJ (2008) The presence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on hellbenders *Cryptobranchus alleganiensis* in the Ozark highlands. Herpetol Rev 39:443–444
- ✦ Burgmeier NG, Sutton TM, Williams RN (2011a) Spatial ecology of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Herpetologica 67:135–145
- ✦ Burgmeier NG, Unger SD, Meyer JL, Sutton TM, Williams RN (2011b) Health and habitat quality assessment for the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana, USA. J Wildl Dis 47:836–848
- ✦ Carey C, Bruzgul JE, Livo LJ, Walling ML and others (2006) Experimental exposures of boreal toads (*Bufo boreas*) to a pathogenic chytrid fungus (*Batrachochytrium dendrobatidis*). EcoHealth 3:5–21
- ✦ 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:e44821
- ✦ Cunningham AA (1996) Disease risks of wildlife translocations. Conserv Biol 10:349–353
- ✦ Da Silva Neto JG, Sutton WB, Spear SF, Freake MJ, Kéry M, Schmidt BR (2020) Integrating species distribution and occupancy modeling to study hellbender (*Cryptobranchus alleganiensis*) occurrence based on eDNA surveys. Biol Conserv 251:108787
- ✦ Davis AK, Hopkins WA (2013) Widespread trypanosome infections in a population of eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in Virginia, USA. Parasitol Res 112:453–456
- Dean N, Ossiboff R, Bunting E, Schuler K, Rothrock A, Roblee K (2016) The eastern hellbender and *Batrachochytrium dendrobatidis* (Bd) in western New York. In: Proc 65<sup>th</sup> Int Conf Wildlife Disease Association, 31 July–5 August 2016, Cortland, NY. Wildlife Disease Association, Lawrence, KS, p 151 (Abstract)
- ✦ Dickens MJ, Delehanty DJ, Romero LM (2010) Stress: an inevitable component of animal translocation. Biol Conserv 143:1329–1341
- Dodd CK, Seigel RA (1991) Relocation, repatriation, and translocation of amphibians and reptiles: Are they conservation strategies that work? Herpetologica 47:336–350
- Eskew EA, Todd BD, Hopkins WA (2014) Extremely low prevalence of *Batrachochytrium dendrobatidis* infection in eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in Southwest Virginia, USA. Herpetol Rev 45:425–427
- ✦ Foster RL, Mcmillan AM, Roblee KJ (2009) Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York State. J Herpetol 43:579–588
- ✦ Freake MJ, DePerno CS (2017) Importance of demographic surveys and public lands for the conservation of eastern hellbenders *Cryptobranchus alleganiensis alleganiensis* in southeast USA. PLOS ONE 12:e0179153
- ✦ Freake M, O'Neill E, Unger S, Spear S, Routman E (2018) Conservation genetics of eastern hellbenders *Cryptobranchus alleganiensis alleganiensis* in the Tennessee Valley. Conserv Genet 19:571–585
- Gascon C, Collins JP, Moore RD, Church DR, McKay JE, Mendelson JR III (eds) (2007) Amphibian conservation action plan. IUCN/SSC Amphibian Specialist Group, Gland
- ✦ Gates JE, Stouffer RH, Stauffer JR Jr, Hocutt CH (1985) Dispersal patterns of translocated *Cryptobranchus alleganiensis* in a Maryland stream. J Herpetol 19:436–438
- ✦ Germano JM, Bishop PJ (2009) Suitability of amphibians and reptiles for translocation. Conserv Biol 23:7–15
- Gonynor JL, Yabsley MJ, Jensen JB (2011) A preliminary survey of *Batrachochytrium dendrobatidis* exposure in hellbenders from a stream in Georgia, USA. Herpetol Rev 42:58–59
- Graham SP, Soehren EC, Cline GR, Schmidt CM and others (2011) Conservation status of hellbenders (*Cryptobranchus alleganiensis*) in Alabama, USA. Herpetol Conserv Biol 6:242–249
- ✦ Griffiths RA, Pavajeau L (2008) Captive breeding, reintroduction, and the conservation of amphibians. Conserv Biol 22:852–861
- ✦ Hanlon SM, Lynch KJ, Kerby J, Parris MJ (2015) *Batrachochytrium dendrobatidis* exposure effects on foraging efficiencies and body size in anuran tadpoles. Dis Aquat Org 112:237–242
- ✦ Hardman RH, Sutton WB, Irwin KJ, McGinnity D and others (2020a) Geographic and individual determinants of important amphibian pathogens in hellbenders (*Cryptobranchus alleganiensis*) in Tennessee and Arkansas, USA J Wildl Dis 56:803–814
- ✦ Hardman RH, Irwin KJ, Sutton WB, Miller DL (2020b) Evaluation of severity and factors contributing to foot lesions in endangered Ozark hellbenders, *Cryptobranchus alleganiensis bishopi*. Front Vet Sci 7:34

- Harris RN, Brucker RM, Walke JB, Becker MH and others (2009) Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *ISME J* 3: 818–824
- ✦ Hillis RE, Bellis ED (1971) Some aspects of the ecology of the hellbender, *Cryptobranchus alleganiensis alleganiensis*, in a Pennsylvania stream. *J Herpetol* 5:121–126
- ✦ Hime PM, Briggler JT, Reece JS, Weisrock DW (2019) Genomic data reveal conserved female heterogamety in giant salamanders with gigantic nuclear genomes. *G<sup>3</sup>: Genes, Genomes, Genetics* 9:3467–3476
- Jachowski CMB, Hopkins WA, Angermeier PL, Belden LK, Millsbaugh JJ, Walters JR (2016) Effects of land use on hellbenders (*Cryptobranchus alleganiensis*) at multiple levels and efficacy of artificial shelters as a monitoring tool. PhD dissertation, Virginia Tech, Blacksburg, VA
- ✦ Kinney VC, Heemeyer JL, Pessier AP, Lannoo MJ (2011) Seasonal pattern of *Batrachochytrium dendrobatidis* infection and mortality in *Lithobates areolatus*: affirmation of Vredenburg's '10,000 zoospore rule'. *PLOS ONE* 6:e16708
- ✦ Kock RA, Woodford MH, Rossiter PB (2010) Disease risks associated with the translocation of wildlife. *Rev Sci Tech* 29:329–350
- Kocher MC (2019) Release of captive-raised eastern hellbenders (*Cryptobranchus alleganiensis*) to test the success of a chytrid vaccine and new cage design. MA thesis, Buffalo State College, Buffalo, NY
- ✦ Kraus BT, McCallen EB, Williams RN (2017) Evaluating the survival of translocated adult and captive-reared, juvenile eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*). *Herpetologica* 73:271–276
- ✦ Kueneman JG, Woodhams DC, Harris R, Archer HM, Knight R, McKenzie VJ (2016) Probiotic treatment restores protection against lethal fungal infection lost during amphibian captivity. *Proc R Soc B* 283:20161553
- ✦ Lambert MR, Womack MC, Byrne AQ, Hernández-Gómez O and others (2020) Comment on 'Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity'. *Science* 367:eaay1838
- ✦ Lips KR, Brem F, Brenes R, Reeve JD and others (2006) Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc Natl Acad Sci USA* 103:3165–3170
- ✦ Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219–227
- ✦ Mathews F, Moro D, Strachan R, Gelling M, Buller N (2006) Health surveillance in wildlife reintroductions. *Biol Conserv* 131:338–347
- Mayasich J, Grandmaison D, Phillips C (2003) Eastern hellbender status assessment report. NRRI/TR-2003/09. US Fish and Wildlife Service, Fort Snelling, MN
- Mazerolle MJ (2019) Package 'AICcmodavg'. R package version 2.2-2. <https://cran.r-project.org/web/packages/AICcmodavg/index.html>
- ✦ McKenzie VJ, Bowers RM, Fierer N, Knight R, Lauber CL (2012) Co-habiting amphibian species harbor unique skin bacterial communities in wild populations. *ISME J* 6:588–596
- ✦ Narayan E, Hero JM (2011) Urinary corticosterone responses and haematological stress indicators in the endangered Fijian ground frog (*Platymanthis vitiana*) during transportation and captivity. *Aust J Zool* 59:79–85
- ✦ Narayan EJ, Molinia FC, Kindermann C, Cockrem JF, Hero JM (2011) Urinary corticosterone responses to capture and toe-clipping in the cane toad (*Rhinella marina*) indicate that toe-clipping is a stressor for amphibians. *Gen Comp Endocrinol* 174:238–245
- ✦ Nickerson MA, Briggler JT (2007) Harvesting as a factor in population decline of a long-lived salamander; the Ozark hellbender, *Cryptobranchus alleganiensis bishopi* Grobman. *Appl Herpetol* 4:207–216
- ✦ Nickerson MA, Mays CE (1973) A study of the Ozark hellbender *Cryptobranchus alleganiensis bishopi*. *Ecology* 54:1164–1165
- ✦ Parris MJ, Beaudoin JG (2004) Chytridiomycosis impacts predator-prey interactions in larval amphibian communities. *Oecologia* 140:626–632
- Pessier AP, Mendelson JR (eds) (2010) A manual for control of infectious diseases in amphibian survival assurance colonies and reintroduction programs. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, MN
- ✦ Peterson JD, Steffen JE, Reinert LK, Cobine PA, Appel A, Rollins-Smith L, Mendonça MT (2013) Host stress response is important for the pathogenesis of the deadly amphibian disease, chytridiomycosis, in *Litoria caerulea*. *PLOS ONE* 8:e62146
- ✦ Piotrowski JS, Annis SL, Longcore JE (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96:9–15
- ✦ Pounds JA, Bustamante MR, Coloma LA, Consuegra JA and others (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439: 161–167
- ✦ Reeder NM, Pessier AP, Vredenburg VT (2012) A reservoir species for the emerging amphibian pathogen *Batrachochytrium dendrobatidis* thrives in a landscape decimated by disease. *PLOS ONE* 7:e33567
- Regester KJ, Simpson H, Chapman EJ, Petokas PJ (2012) Occurrence of the fungal pathogen *Batrachochytrium dendrobatidis* among eastern hellbender populations (*Cryptobranchus a. alleganiensis*) within the Allegheny-Ohio and Susquehanna River Drainages, Pennsylvania, USA. *Herpetol Rev* 43:90–93
- ✦ Rollins-Smith LA, Doersam JK, Longcore JE, Taylor SK, Shamblin JC, Carey C, Zasloff MA (2002) Antimicrobial peptide defenses against pathogens associated with global amphibian declines. *Dev Comp Immunol* 26:63–72
- ✦ Rothermel BB, Walls SC, Mitchell JC, Dodd CK Jr and others (2008) Widespread occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in the southeastern USA. *Dis Aquat Org* 82:3–18
- RStudio Team (2020) RStudio: integrated development for R. RStudio, Boston, MA
- ✦ Scheele BC, Hunter DA, Grogan LF, Berger LEE and others (2014) Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians. *Conserv Biol* 28:1195–1205
- ✦ Scheele BC, Pasmans F, Skerratt LF, Berger L and others (2019) Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science* 363: 1459–1463
- ✦ Scheele BC, Pasmans F, Skerratt LF, Berger L and others (2020) Response to Comment on "Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity". *Science* 367:eaay2905
- ✦ Seeley KE, D'Angelo M, Gowins C, Greathouse J (2016) Prevalence of *Batrachochytrium dendrobatidis* in eastern hellbender (*Cryptobranchus alleganiensis*) populations in West Virginia, USA. *J Wildl Dis* 52:391–394

- Smith BG (1907) The life history and habits of *Cryptobranchus alleganiensis*. Biol Bull (Woods Hole) 13: 5–39
- Smith KF, Acevedo-Whitehouse K, Pedersen AB (2009) The role of infectious diseases in biological conservation. Anim Conserv 12:1–12
- Souza MJ, Gray MJ, Colclough P, Miller DL (2012) Prevalence of infection by *Batrachochytrium dendrobatidis* and *Ranavirus* in eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in eastern Tennessee. J Wildl Dis 48:560–566
- Stockwell M, Clulow S, Clulow J, Mahony M (2008) The impact of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* on a green and golden bell frog *Litoria aurea* reintroduction program at the Hunter Wetlands Centre Australia in the Hunter Region of NSW. Aust Zool 34:379–386
- Stockwell MP, Clulow J, Mahony MJ (2010) Host species determines whether infection load increases beyond disease-causing thresholds following exposure to the amphibian chytrid fungus. Anim Conserv 13:62–71
- Stouffer RH, Gates JE, Hocutt CH, Stauffer JR (1983) Surgical implantation of a transmitter package for radio-tracking endangered hellbenders. Wildl Soc Bull 11:384–386
- Suarez MB, Ewen JG, Groombridge JJ, Beckmann K and others (2017) Using qualitative disease risk analysis for herpetofauna conservation translocations transgressing ecological and geographical barriers. EcoHealth 14: 47–60
- Tominaga A, Irwin KJ, Freake MJ, Suzuki K, Goka K (2013) *Batrachochytrium dendrobatidis* haplotypes on the hellbender *Cryptobranchus alleganiensis* are identical to global strains. Dis Aquat Org 102:181–186
- Unger SD, Williams LA, Groves JD, Lawson CR, Humphries WJ (2017) Anthropogenic associated mortality in the eastern hellbender. Southeast Nat 16:N9–N13
- USFWS (US Fish and Wildlife Service) (2018) Species status assessment report for the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*). US Fish and Wildlife Service Midwest Region, Bloomington, MN
- Utrup J, Mitchell K (2008) The Ozark hellbender: out from under a rock. Endang Species Update 25:S14
- Vredenburg VT, Knapp RA, Tunstall TS, Briggs CJ (2010) Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proc Natl Acad Sci USA 107:9689–9694
- Walker SF, Bosch J, James TY, Litvintseva AP and others (2008) Invasive pathogens threaten species recovery programs. Curr Biol 18:R853–R854
- Weinstein SB (2009) An aquatic disease on a terrestrial salamander: individual and population level effects of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, on *Batrachoseps attenuatus* (Plethodontidae). Copeia 2009:653–660
- Wheeler BA, Prosen E, Mathis A, Wilkinson RF (2003) Population declines of a long-lived salamander: a 20+ year study of hellbenders, *Cryptobranchus alleganiensis*. Biol Conserv 109:151–156
- Williams LA, Groves JD (2014) Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in eastern hellbenders (*Cryptobranchus a. alleganiensis*) in western North Carolina, USA. Herpetol Conserv Biol 9: 454–467
- Williams RD, Gates JE, Hocutt CH, Taylor GJ (1981) The hellbender: a nongame species in need of management. Wildl Soc Bull 9:94–100

Editorial responsibility: Douglas Woodhams,  
Boston, Massachusetts, USA  
Reviewed by: 2 anonymous referees

Submitted: November 16, 2020  
Accepted: April 22, 2021  
Proofs received from author(s): June 28, 2021