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## Evaluating the Survival of Translocated Adult and Captive-reared, Juvenile Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*)

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**ABSTRACT:** Recent amphibian declines have been addressed with a variety of conservation tools, including translocations. It is important to evaluate the success of ongoing amphibian conservation programs. In Indiana, USA, because of the documented decline of the Eastern Hellbender (*Cryptobranchus a. alleganiensis*) population, translocations of wild-captured and captive-reared individuals have been implemented as a management strategy. Our study was developed to estimate the annual survivorship of translocated versus resident Eastern Hellbenders and to compare the efficacy of translocating wild adults and captive-reared juveniles. Adult residents, and translocated adult and juvenile subjects, were implanted with radio transmitters in a staggered entry design and tracked for up to 2 yr. A Cox proportional hazards model indicated that captive-reared, juvenile hellbenders had lower annual survival rates than did either resident or translocated adults. The relatively high survival rates of all groups indicated the potential utility of both types of translocations as a tool to increase local densities, although there might be room for improvement of the survival rates for captive-reared juveniles through either increased rearing periods or environmental conditioning prior to release.

**Key words:** Amphibian declines; Augmentation; Caudata; Radio-telemetry; Salamander

AMPHIBIAN declines have been severe and far-reaching (Stuart et al. 2004; McCallum 2007; Adams et al. 2013), with a wide variety of causes including habitat loss, introduced species, disease, and climate change (Dodd 1997; Daszak et al. 2003; Kats and Ferrer 2003; Lips et al. 2008). A number of conservation measures targeting amphibians have been undertaken including translocations to augment existing populations and to reintroduce populations to areas where they were previously extirpated (Dodd and Seigel 1991; Griffiths and Pavajeau 2008; Germano and Bishop 2009). The outcomes of amphibian translocations have varied greatly by taxa, and failures are common although decreasing in prevalence over time (Dodd and Seigel 1991; Griffiths and Pavajeau 2008; Germano and Bishop 2009). Whereas it is inherently difficult to define and measure success in the context of these programs, an important first step for the re-establishment of an effective population is the survival of translocated individuals (Germano and Bishop 2009).

One amphibian species currently undergoing extensive conservation measures in the United States is Eastern Hellbenders (*Cryptobranchus a. alleganiensis*; Briggler et al. 2010; Williams et al. 2015). Eastern Hellbender populations have undergone precipitous declines in many parts of their range within recent decades (Mayasich et al. 2003; Wheeler et al. 2003). The historic range of the Eastern Hellbender in Indiana, USA, included most of the Ohio River and lower Wabash River drainages (Petranka 1998) but is now restricted to the Blue River drainage (Kern 1984; Burgmeier et al. 2011a). Individual densities in Indiana's Eastern Hellbender populations are critically low, increasing

the likelihood of inbreeding depression, genetic bottlenecks, and extirpation via stochastic events (Tanaka 2000). To alleviate Eastern Hellbender population declines, the Indiana Department of Natural Resources (IDNR) is now integrating Eastern Hellbender translocations within the management strategy for this species.

Many factors, such as the demography of release groups and habitat quality within release sites, are known to influence translocation success (Wolf et al. 1996; Griffiths and Pavajeau 2008; Germano and Phillips 2009). Age and captive-rearing status are potentially important factors because both smaller size and inexperience might reduce survival rates in hellbenders (Bodinof et al. 2012). Sex may also be an important factor in survival of Eastern Hellbenders. Because home range size is greater in females than in males in the Indiana population, particularly during the breeding season (Burgmeier et al. 2011b), females might be more vulnerable to threats such as predation. Site quality also appears to be an important factor in the survival probability of translocated hellbenders (Bodinof et al. 2012). Quantifying differences in survival rates might guide decisions in future hellbender translocation events, specifically in terms of release group demographics and release site selection.

The goal of our study was to estimate post release survival rates via radio telemetry in order to evaluate the efficacy of translocating wild, adult Eastern Hellbenders and captive-reared, juvenile Eastern Hellbenders. Specifically, we wanted to (1) determine the importance of age, translocation status, sex, and site location on survival rates of monitored Eastern Hellbenders; and (2) estimate the survival rates of

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TABLE 1.—Details of individual Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) released at Site 1 in the Blue River, Indiana, to compare the fates of wild, adult residents and wild, translocated adults. Right-censored individuals could not be located at the end of the study because of either transmitter failure or dispersal from the study site.

Individual	Release date	Resident status	Age	Sex	Fate as of August 2013
1	14 June 2011	Resident	Adult	Male	Alive
2	16 June 2011	Resident	Adult	Male	Alive
3	13 July 2011	Resident	Adult	Male	Alive
4	14 July 2011	Resident	Adult	Female	Alive
5	15 July 2011	Resident	Adult	Female	Alive
6	27 July 2011	Resident	Adult	Male	Right censored
7	30 July 2011	Resident	Adult	Female	Dead
8	30 July 2011	Resident	Adult	Female	Dead
9	3 August 2011	Resident	Adult	Female	Alive
10	15 August 2011	Resident	Adult	Female	Right censored
11	11 October 2011	Resident	Adult	Male	Right censored
12	3 August 2011	Translocated	Adult	Female	Right censored
13	3 August 2011	Translocated	Adult	Male	Right censored
14	9 August 2011	Translocated	Adult	Male	Alive
15	9 August 2011	Translocated	Adult	Male	Alive
16	11 August 2011	Translocated	Adult	Male	Alive
17	16 August 2011	Translocated	Adult	Male	Alive
18	16 August 2011	Translocated	Adult	Male	Right censored
19	17 August 2011	Translocated	Adult	Female	Dead
20	25 June 2012	Translocated	Adult	Male	Dead
21	18 July 2012	Translocated	Adult	Male	Alive
22	18 July 2012	Translocated	Adult	Female	Right censored

groups determined by previous analysis to be statistically different.

## MATERIALS AND METHODS

### Study Area and Site Selection

The Blue River originates in Washington County, Indiana and flows south through Harrison County prior to converging with the Ohio River in Crawford County. This study included two sites in the southern portion of the Blue River. Both sites (hereafter Site 1 and Site 2) are ~500 m in length and contain large stretches of gravel and cobble substrate and flat, boulder-sized rocks that hellbenders use as shelter and nesting habitat (Hillis and Bellis 1971). Both sites were previously ranked as suitable habitat according to the criteria listed in Burgmeier et al. (2011b). The main factor influencing site selection was high initial densities of Eastern Hellbenders (Burgmeier et al. 2011a); however, close site proximity (~17 river km), ease of access, and low frequency of human use were also considered as important attributes.

### Field Methods

We tracked and located Eastern Hellbenders via radio telemetry at both study sites from June 2011–July 2013. Site 1 contained 11 resident adult Eastern Hellbenders and 11 adult Eastern Hellbenders that were translocated from other parts of the Blue River (Table 1). The translocated individuals were moved from low-density areas (they were often the only known individuals present at each site) in order to increase breeding opportunities at Site 1. Site 2 contained 10 resident adult Eastern Hellbenders and 10 captive-reared, juvenile Eastern Hellbenders (Table 2). These individuals were introduced to compare the efficacy of translocating naïve juvenile hellbenders instead of experienced adults. Sample subjects underwent staggered entry into the study (Tables 1, 2).

Resident hellbenders were captured by hand or net via rock-lifting surveys. Upon capture, we recorded the total

length (TL; cm), snout–vent length (SVL; cm), and mass (g) of all individuals. We conducted surgeries in the field on all adults captured in the Blue River. Translocated adults were transported to Site 1 prior to surgery via the method described in Kenison et al. (2016). All hellbenders captured in the Blue River were adults between 422 g and 1218 g (mean  $\pm$  1 SE =  $16 \pm 33.5$  g) at the time of capture. Adults were implanted with a 13-g, 50  $\times$  11 mm, SI-2 model transmitter with whip antenna (Holohil Systems Ltd.) and a Biomark 134.2 kHz ISO passive integrated transponder (PIT) tag (23 mm long  $\times$  3.85 mm diameter; mass = 616 mg) following the surgical methods described in (Burgmeier et al. 2011b).

Translocated juvenile Eastern Hellbenders were captive-reared from a single clutch collected from Buffalo Creek, West Virginia, USA and raised at the Fort Worth Zoo, Fort Worth, Texas, USA. During June 2011, we transported 3.5-yr-old individuals ( $n = 18$ ) to Purdue University's Aquaculture Research Laboratory, West Lafayette, Indiana. All juveniles were maintained in a flow-through system with a flow rate of 12-L/min and a water temperature of ~14°C. The system consisted of 10 interconnected, 150-L glass aquaria. We housed two individuals per tank and used the final tank to house crayfish. We lined the tanks with gravel and cobble substrates and provided two flat, flagstone slabs for refugia within each tank. Juveniles were fed ad libitum with red worms (*Eisenia fetida*), black worms (*Lumbriculus variegatus*), and crayfish (*Orconectes rusticus*). We monitored the monthly growth of all individuals including TL (cm), SVL (cm), and mass (g).

In September 2012, the 10 largest individuals were examined via laparoscope by veterinarians at Purdue University to determine sex and implanted with radio transmitters and PIT tags. Prior to surgery, subjects were anesthetized with tricaine methanesulfonate (MS-222) buffered with baking soda. Three individuals were larger than their peers, weighing 243–297 g ( $\bar{X} = 268 \pm 15.8$  g) and

TABLE 2.—Details of individual Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) released at Site 2 along the Blue River, Indiana to compare the fates of wild, adult residents and captive-reared, translocated juveniles. Right-censored individuals could not be located at the end of the study because of either transmitter failure or dispersal from the study site.

Individual	Release date	Resident status	Age	Sex	Fate as of August 2013
1	18 July 2011	Resident	Adult	Female	Alive
2	18 July 2011	Resident	Adult	Female	Right censored
3	18 July 2011	Resident	Adult	Female	Dead
4	20 July 2011	Resident	Adult	Female	Right censored
5	21 July 2011	Resident	Adult	Male	Alive
6	22 July 2011	Resident	Adult	Male	Right censored
7	24 July 2011	Resident	Adult	Male	Dead
8	10 October 2011	Resident	Adult	Male	Alive
9	10 October 2011	Resident	Adult	Male	Alive
10	28 June 2012	Resident	Adult	Male	Alive
11	8 October 2012	Translocated	Juvenile	Male	Dead
12	8 October 2012	Translocated	Juvenile	Male	Alive
13	8 October 2012	Translocated	Juvenile	Female	Alive
14	8 October 2012	Translocated	Juvenile	Female	Right censored
15	8 October 2012	Translocated	Juvenile	Male	Right censored
16	8 October 2012	Translocated	Juvenile	Male	Right censored
17	8 October 2012	Translocated	Juvenile	Female	Right censored
18	8 October 2012	Translocated	Juvenile	Male	Dead
19	8 October 2012	Translocated	Juvenile	Male	Dead
20	8 October 2012	Translocated	Juvenile	Female	Dead

were implanted with the same transmitters and PIT tags as the adult Hellbenders. The remaining seven individuals weighed between 158–240 g ( $\bar{X} = 197 \pm 10.0$  g). These smaller subjects were implanted with a 9-g, 33 × 11 mm, SI-2 model transmitter with whip antenna (Holohil Systems, Ltd) and the same PIT tags as the adult hellbenders. Following surgery, we placed each subject into separate 75-L aquaria with cool, well-oxygenated water and observed their behavior. When individuals displayed normal behavioral patterns including righting response, exploratory behavior, and a reasonable attempt to escape capture, we returned them to their original tanks. The juveniles were held in captivity for an additional month post surgery to monitor healing and suture resorption. There was no evidence of suture dehiscing among juveniles during this period.

Residents were released under their initial capture rock. Translocated subjects were released into artificial nest rocks placed in the river to augment existing habitat and provide nesting locations (Briggler and Ackerson 2012). During their release, all individuals were placed within soft-release enclosures to ensure they would not disperse from the study area. The enclosures consisted of a polyvinyl chloride (PVC) cube covered with seine netting (6.35 mm mesh). We constructed the frame using PVC pipe (25.4 mm diameter) and 90° PVC side outlet elbows. The final cube dimensions were 76 × 76 × 61 cm (length × width × height). Multiple holes (6.35 mm in diameter) were drilled through the PVC frame to alleviate buoyancy of the enclosure. The seine netting was secured with zip-ties to the PVC frame to complete the enclosure. The edges of the enclosures were buried with existing substrate to help stabilize the enclosure as well as to prevent individuals from escaping. Enclosures were removed 48–60 hr post release and Eastern Hellbenders were then monitored via radiotelemetry. Three individuals escaped their enclosures within 24 hr and were subsequently monitored.

Eastern Hellbenders were located by radiotelemetry with a homing technique (White and Garrott 1990) using a two-element H antenna coupled with a TRW-1000 telemetry

receiver (Wildlife Materials, Inc.). We tracked and located all individuals, conditions permitting, three times weekly during the summer and fall (June–November) and at least monthly during the rest of the year (December–May). Once we located an individual, we recorded a Universal Transverse Mercator (UTM, Zone 16N) coordinate using a Garmin global positioning system (GPS) unit (resolution  $\pm 4$  m; Rino 110, Garmin). We considered directed, upstream movements evidence of survival but relied on site surveys during Summer 2013 to confirm survivorship (Olson et al. 2013). Radio transmitters were not removed upon the completion of the study.

#### Statistical Analysis

On account of the staggered-entry of individuals into the study, we used a Cox proportional hazards model to relate covariates to survival time and to estimate baseline annual survival rates (Cox 1972). We conducted all statistical analyses in R v3.0.2 (R Core Team 2013) using the *coxph* function (Therneau 2016). Subjects who disappeared from release sites or experienced transmitter failure prior to the end of the study were right-censored on the first day that they were not observed in the field. Mortalities were coded as events on the date that either hellbender remains or an isolated transmitter was discovered. We incorporated four categorical, time-independent, explanatory covariates into our analysis: age class (juvenile or adult), resident status (resident or translocated), site (Site 1 or Site 2), and sex (male or female). Because of sample size limitations, we only examined the main effects of covariates. We used backwards selection and likelihood ratio tests to evaluate predictor significance ( $\alpha < 0.05$ ). We used the *survfit* function (Therneau 2016) to estimate survival rates over time for groups that were deemed statistically independent based on the final model.

#### RESULTS

At Site 1, 22 individuals entered the study between July 2011 and July 2012 (Table 1). At the end of the study in

August 2013, 11 individuals were coded as alive at Site 1 (Table 1). Seven individuals from Site 1 were right-censored from 52 to 680 d ( $\bar{X} = 454$  d) after entry into the study. We coded four individuals as mortalities at Site 1 (Table 1). We discovered the carcass of a single individual after 649 d in the study. For all other deaths, we recovered only transmitters. One transmitter at the site was discovered 7 d after surgery and might have been the result of dehiscence, but we coded it as a mortality to ensure a conservative survival rate estimation. Two other transmitters were discovered at the site 456 d and 667 d post release, respectively, and were likely the result of true mortality.

At Site 2, 20 individuals entered the study between July 2011 and October 2012. At the end of the study in August 2013, seven individuals were coded as alive at Site 2 (Table 2). Seven individuals from Site 2 were right-censored from 195 to 725 d ( $\bar{X} = 410$  d) after entry into the study. We coded six individuals as mortalities at Site 2 (Table 2). We discovered the carcass of a single individual after 459 d in the study. For all other coded mortalities, only the transmitter was recovered and these occurred between 195 and 695 d post release ( $\bar{X} = 376$  d). Again, these were likely true mortalities because they occurred so late in the study. One juvenile was removed from the study 6 d after initial release because of an incidental observation of suture rupture; this subject was returned to the Purdue University's Aquaculture Research Laboratory for 10 d of treatment. Another juvenile left the site 3 d after the soft-release enclosure was removed. The individual was captured  $\sim 1.3$  km downstream from its original location and returned to the site. We coded the start date of both individuals as the day they returned to the study site.

Out of the 10 coded mortalities, 4 occurred in resident adults, 2 occurred in translocated adults, and 4 occurred in captive-reared juveniles. The only significant covariate in the final model was age with a coefficient value ( $\pm 1$  SE) of  $-2.95 \pm 1.14$  ( $P = 0.01$ ). At 365 d, the cumulative estimated survival ( $\pm 95\%$  CI) for juveniles was 0.526 (0.263–1) and the cumulative estimated survival for adults (residents and translocated subjects) was 0.967 (0.906–1). At 730 d, the cumulative estimated survival rate for adults was 0.739 (0.570–0.957). The 2-yr survival rate was not estimated for juveniles because of their late entry into the study.

#### DISCUSSION

Translocations are an important tool for augmenting and repatriating imperiled amphibian species (Griffiths and Pavajeau 2008; Germano and Bishop 2009). Translocations of adults can increase local densities and encourage breeding opportunities (Deredec and Courchamp 2007). Headstarting can increase survival for particularly vulnerable age classes (Haskell et al. 1996; Alberts 2007) and, when followed by successful translocations, increase recruitment rates in populations (George et al. 2009). This is the first study to evaluate the survival of wild, translocated adult and captive-reared, juvenile Eastern Hellbenders using radiotelemetry. This project is particularly well suited to assess the survivorship of translocated adult Eastern Hellbenders because we can compare the survivorship of translocated adults directly with existing data on this population prior to translocations (Olson et al. 2013).

Despite the use of soft-release techniques, careful surgical procedures, and extended monitoring, 10 presumed mortalities were documented over the course of our study. We have limited evidence of the potential causes of mortality in our monitored individuals. Mortalities associated with surgical procedures appeared rare. In our documented adult mortalities, we had one transmitter discovered 7 d post surgery which was likely surgery related. However, all other adult mortalities occurred more than a year post surgery. The first documented juvenile mortality did not occur until 195 d after release. In a study that followed a cohort of similar age, 2 of 13 mortalities occurred following surgeries and an additional five occurred within 30 d post release, with three of the mortalities displaying post release dehiscence (Bodinof et al. 2012). We had one juvenile dehiscence post release, but we were able to capture and rehabilitate the individual. A previous survivorship study of hellbenders in Indiana found similarly low rates of surgery-related mortality (Olson et al. 2013). While it is impossible to discount long-term survival impacts of transmitter implantation on our study population, short-term impacts appeared limited. In our study, both of the discovered hellbender carcasses were found out of the water above steep embankments and two transmitters were discovered within mammalian bank dens, indicating that mammalian predators are a likely cause of mortality in our population.

Subject age was the only significant covariate in our Cox proportional hazards model. Translocated juveniles displayed a lower survival rate than did adults; however, an annual survival rate of 0.526 is comparable to the lower range of survival estimates for a similar cohort of naïve, juvenile Ozark Hellbenders (*C. a. bishopi*; Bodinof et al. 2012). It is impossible to precisely identify the reason why juvenile survival was lower than adult survival in our study. As with other salamanders, hellbender survival is likely lower in younger age classes (Sagar et al. 2007; Lee et al. 2012). Even when they persist through the egg and larval stages, survival rates of juvenile hellbenders might be lower than adults because of flooding displacement or increased predation (Nickerson and Mays 1973; Taber et al. 1975). Besides being young, our subjects were also naïve, as they were hatched and raised in captive conditions until their release. Captive-rearing techniques might be detrimental to overall survival probability if they fail to prepare translocated individuals for the challenges associated with life in natural conditions (Shepherdson 1994). Regardless of the reason for the low annual survivorship, it is important to increase survival in this cohort because they will not influence future population success until reaching sexual maturity at 5–7 yr old (Taber et al. 1975).

Given the mixed results of previous herpetofauna translocations (Dodd and Seigel 1991; Griffiths and Pavajeau 2008; Germano and Bishop 2009), we expected translocated adults to have lower survival rates than would resident adults; however, rates for the two groups were similar. Furthermore, increasing local site densities did not decrease adult survivorship compared to baseline levels (Olson et al. 2013). This suggests that availability of quality habitat was not a factor in our study, contrary to the results of a previous survival study concerning hellbenders (Bodinof et al. 2012). A thorough study of the Blue River population and habitat

prior to any conservation actions likely assisted with the appropriate selection of sites for translocation efforts (Burgmeier et al. 2011c). The ability of the sites to support translocated individuals might also be attributable to low initial hellbender densities (Burgmeier et al. 2011a). Even after doubling the number of individuals at both sites, site densities were still well below those reported historically within the Blue River (Kern 1984).

We also failed to detect an anticipated effect of sex on survival rates. A recent survey of hellbenders in the Blue River revealed a skewed sex ratio of more than 2:1 in favor of males (Burgmeier et al. 2011a). Because sex ratios in the Blue River were once nearly even (Kern 1984), we expected to see higher male survivorship in our population. It is possible we failed to see this expected effect because of our small sample size. Even a relatively small difference in survivorship, with an effect size too small to detect with our current statistical power, could skew sex ratios over time because hellbenders are a long-lived species. However, there is also a possible alternative explanation for the lack of observed pattern. Female hellbenders in Indiana have larger linear home ranges than do males during the breeding season (Burgmeier et al. 2011b), and increased movement during breeding events often leads to higher mortality (Clevenger et al. 2001; Iosif et al. 2013). Because the sites within this study represented relatively high-density sites within the Blue River, it is possible that females in this study did not have to move as far for mating opportunities, resulting in more-commensurate survival rates.

The population demography of Eastern Hellbenders in Indiana has been studied for decades and reveals that the population consists of a single older age class with little to no recruitment (Kern 1984; Burgmeier et al. 2011b). The translocation of adults in this study appeared to be an effective method of bolstering local reproduction, as three nests were found in Site 1 in the 4 yr immediately following the adult translocations. Based on the comparable survival rates of translocated and resident adult hellbenders, we recommend this method as a means of increasing local densities and encouraging reproduction. However, translocating adult hellbenders within rivers will inevitably have diminishing returns in rapidly aging populations. The release of captive-reared juveniles might serve as a means to establish younger age classes, particularly if annual survival can be increased from current rates. A recent population viability analysis for Eastern Hellbenders in Indiana revealed that increased survivorship of younger age classes could cause a significant reduction in the probability of extinction within the state (Unger et al. 2013). Herein, we have successfully established baseline survival data for captive-reared juveniles that can be used for future population augmentations and survival assessments. A thorough analysis of movement patterns and habitat use in the study subjects might help elucidate the survival patterns described within this study. Further study should focus on whether survivorship can be increased by longer rearing times or by modifying rearing techniques within a captive setting to mimic more-natural conditions. Only continued monitoring of translocation efforts can demonstrate the long-term success of these programs in Indiana.

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