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Comparing the Effects of Environmental Enrichment, Seasonality, and Soft Release on Site Retention and Survivorship of Captive-reared Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*)

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Abstract: Translocations of captive-reared animals are commonly used to stabilize declining wildlife populations. However, captive-reared animals are often raised in conditions dissimilar to their release sites and lacking natural characteristics, which could alter movement patterns and postrelease survivorship. These patterns can be further altered by season of release and soft-release conditions. We reared juvenile Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) for 18 mo in captivity in one of two conditions: a control condition with low-velocity water flow (unconditioned) or a treatment condition with moving water (conditioned) that simulated natural flow velocities at their intended release site. We divided 4-yr-old Hellbenders (*n* ¼ 118) into six treatment groups to determine the effects of release season (fall or summer), release type (standard soft release or enhanced soft release), and conditioning (unconditioned or conditioned) on the number of days until first movement, release site retention, and survival. In November 2017, we released 80 radio-tagged individuals (40 conditioned and 40 unconditioned) into soft-release structures in the Blue River, Indiana. In July 2018, we released another 38 radio-tagged individuals (18 conditioned and 20 unconditioned) into soft-release structures at the same site. After release, we tracked each individual one to three times weekly for 10 mo (fall release) or 12 mo (summer release). We found that treatment groups released into caged cobble beds (i.e., enhanced soft release) delayed their first movement and had higher release site retention relative to groups released under caged shelter rocks (i.e., standard soft release). We found that conditioning had a positive effect on survival but only in the treatment group released in the summer. By combining techniques and releasing conditioned individuals in the summer using enhanced soft releases, we increased annual survival of captive-reared Hellbenders from a probability of 0.50 (95% confidence interval [CI] ¼ 0.31–0.79) to 0.74 (95% CI ¼ 0.55–0.99). Our results provide important information about techniques that can be adopted across captive-rearing programs to help maximize the conservation success of Eastern Hellbenders.

Key words: Amphibian declines; Augmentation; Caudata; Conditioning; Prerelease training; Radiotelemetry; Reintroduction; Salamanders; Translocation

Translocations are frequently used conservation strategies for restoring declining and extirpated wildlife populations (Brichieri-Colombi and Moehrenschlager 2016; Jachowski et al. 2016). These techniques have been used to restore wildlife populations across a wide range of taxa (Fischer and Lindenmayer 2000; Reading et al. 2013; Soorae 2018). However, despite some successes, these methods are frequently met with mixed results and failure (Griffith et al. 1989; Dodd and Seigel 1991, 2002; Fischer and Lindenmayer 2000; Miller et al. 2014). A common cause of failure for these projects is low postrelease survival of captive-reared individuals (i.e., head-started individuals; Mathews et al. 2005; Blythe et al. 2015). Moreover, poor site retention and a lack of relevant studies from which to draw guidance can significantly decrease the probability of success (Germano and Bishop 2009; Roe et al. 2010; Knox and Monks 2014). To remedy this, some biologists have suggested implementing environmental conditioning, seasonality, and soft-release techniques to improve postrelease survivorship (Reading et al. 2013; Blythe et al. 2015; Tetzlaff et al. 2019a)

Captive-rearing facilities have traditionally focused on rearing animals in sanitary, low-stress conditions bearing little resemblance to the species' natural environment. These head-started animals are physically healthy but often do not exhibit the morphological and behavioral traits necessary for survival in the wild (Snyder et al. 1996; Stoinski et al. 2003; Kelly et al. 2005; Blythe et al. 2015). One strategy to overcome these limitations is environmental conditioning (Mathews et al. 2005; Tetzlaff et al. 2019a,b). Captive-reared (head-started) individuals are exposed to common environmental factors, such as naturalistic habitat, predators, microbiota, and appropriate food items, with the goal of preparing them for the conditions they will experience postrelease (Kenison and Williams 2018a; Hernandez-Gomez et al. 2019; Tetzlaff et al. 2019a; Kenison et al. 2020). These techniques have altered morphology and behavior in a laboratory setting (Kenison and Williams 2018a,b) and have translated to increased survivorship in some species in the wild (Biggins et al. 1998; Tetzlaff et al. 2019a). Black-footed Ferrets (*Mustela nigripes*) raised in large experimental pens designed to emulate their natural environment had 2.7 times higher survivorship than those raised in traditional cages (Biggins et al. 1998). Postrelease

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recovery rates for Chinook Salmon (*Oncorhynchus tshawytscha*) reared in environmentally enriched, seminatural raceways were higher (48% vs. 38%) than those of salmon reared in traditional raceways (Maynard et al. 1996).

High-volume postrelease dispersal of individuals away from release sites is also cited as a frequent cause of failure for translocation programs (Le Gouar et al. 2012; Tetzlaff et al. 2019a; Berger-Tal et al. 2020). Frequent and/or long-distance movements can increase energy expenditure and exposure to predators, resulting in reduced survivorship (Matthews 2003; Hester et al. 2008; Blythe et al. 2015). For example, translocated, hard-released (i.e., released directly into the wild with no acclimation period) Eastern Box Turtles (*Terrapene carolina carolina*) were reported to move farther and experience significantly higher mortality than resident turtles (Hester et al. 2008).

To help reduce postrelease dispersal and increase site fidelity, some translocation programs have adopted soft-release techniques (i.e., a gradual, stepwise release to the wild) designed to give individuals an opportunity to lower their stress response and acclimate to local conditions (Bright and Morris 1994; Blythe et al. 2015). A study on hatchery-reared Brown Trout (*Salmo trutta*) reported higher postrelease recovery in the stocking area at one site for fish given a 24-hr in-site acclimation period than for those with no acclimation period (Cresswell and Williams 1983). However, the release occurred during very low flow conditions, and no difference was observed between acclimated and unacclimated fish at a second site with higher flow conditions (Cresswell and Williams 1983).

Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are large, fully aquatic salamanders that inhabit rocky, swift-flowing rivers and streams and spend most of their time under cobble and large boulders (Smith 1907; Hillis and Bellis 1971). They have experienced precipitous population declines and are protected by state regulations in most states throughout their range (Mayasich et al. 2003; Wheeler et al. 2003; Burgmeier et al. 2011b). Many conservation organizations have worked with state agencies to implement head-starting and release programs to reestablish or stabilize extirpated and declining populations (IDNR 2017). The success of released captive-reared Hellbenders is variable and is likely related to size of individuals at release, the habitat quality of release sites, and stochastic events. Annual survival estimates of captive-reared Hellbenders large enough to track via radiotelemetry range from 17% to 75% (Bodinof et al. 2012g; Boerner 2014; Kraus et al. 2017). It is important to maximize the survival of released animals to ensure the establishment of viable populations.

For Hellbenders, long-distance upstream and downstream movements have been recorded following release (Bodinof et al. 2012a; McCallen et al. 2018), which can be problematic because high-quality habitat for Hellbenders is often separated by long stretches of low-quality habitat. Soft releases are becoming more common for releases of Hellbenders and are hypothesized to reduce initial dispersal and increase survival rates in translocated individuals (Kraus et al. 2017). Combining soft releases with habitat enhancement is especially promising for Hellbenders, because artificial nest rocks are used when habitat is limited (Jachowski et al. 2020) and are used more often by translocated individuals (McCallen et al. 2018). Boerner (2014) found no differences in movement or survival between captive-reared cohorts of Eastern Hellbenders released under rocks (i.e., hard release), released under rocks with cages (i.e., standard soft release), and released into nest boxes (i.e., enhanced soft release); however, both sample sizes and overall survival rates were low in their study. Furthermore, nest boxes may not represent optimal habitat for captive-reared juvenile Hellbenders, who use artificial shelters at lower rates than translocated adults (McCallen et al. 2018). Because juvenile Hellbenders prefer smaller shelter rocks (McCallen et al. 2018; Hecht et al. 2019) and coarse substrates (Bodinof et al. 2012c; Hecht et al. 2019; Unger et al. 2020b), enhanced soft releases into cobble beds into which they can burrow between the rocks may prove more effective at influencing movement and survival in captive-reared cohorts. A significant time and financial investment is made for each released individual (Berger-Tal et al. 2020), and combining multiple conservation strategies might work synergistically to improve survival among released animals.

Furthermore, the seasonality of releases is also an important consideration for translocation of Eastern Hellbenders. Seasonal variations in both abiotic and biotic factors can affect the probability of translocation success. For example, weather-related threats such as floods, fires, and drought vary seasonally and can cause mass mortality in translocated cohorts (Jachowski et al. 2016). In lotic systems, seasonal increases in precipitation often increases water velocity and flooding, which may cause displacement and mortality in Eastern Hellbenders (Humphries 2005; Nickerson et al. 2007; Unger et al. 2021). The risk of seasonal flooding events may be further complicated by seasonal activity patterns. Eastern Hellbenders have an active season starting in late spring, peaking during a late summer breeding season, and ending in late fall when Eastern Hellbenders become mostly dormant before and throughout winter (Blais 1989; Humphries and Pauley 2000; Humphries 2007; Burgmeier 2011a). Seasonal differences in animal behavior, such as those between fall breeding and winter dormancy, may drive seasonal differences in translocation success, as released individuals instinctively explore their environments or enter dormancy. Additional biotic factors may vary seasonally and affect translocation success in Eastern Hellbenders, including risk of chytrid fungal infection (Sonn et al. 2019), conspecific aggression (Unger et al. 2020a), and predator foraging behavior (Serfass et al. 1990). However, little research has been published directly comparing the success of translocations of Hellbenders between seasons. Bodinof et al. (2012b) reported that juvenile Ozark Hellbenders (*C. a. bishopi*) released in spring, summer, and fall showed no significant differences in survivorship between seasons; however, their study was not specifically designed to assess the timing of releases.

Our study builds upon the work of Kenison and Williams (2018a), who found that rearing Eastern Hellbenders with moving water that mimicked a riverine environment altered tail morphology and improved swim performance (Kenison and Williams 2018a). We sought to determine whether these captive-rearing conditions, as well as release season and soft-release type, affect postrelease survivorship of Eastern Hellbenders. We used a factorial design to determine the

TABLE 1.—Summary of the characteristics of six treatment groups of captive-reared Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) released in the Blue River, Indiana, in 2017 and 2018. Treatment groups are based on release season (fall or summer), release type (standard soft release or enhanced soft release), and conditioning status (unconditioned or conditioned).

Treatment group	Comparison dates	Sample size	Known mortalities	Mean mass (g) ± SE
Fall, standard soft release, unconditioned	1 Nov 2017–1 Sept 2018	20	7	150.0 ± 4.9
Fall, standard soft release, conditioned	1 Nov 2017–1 Sept 2018	20	11	145.3 ± 5.3
Fall, enhanced soft release, unconditioned	1 Nov 2017–1 Sept 2018	20	6	143.4 ± 5.1
Fall, enhanced soft release, conditioned	1 Nov 2017–1 Sept 2018	20	10	136.1 ± 6.1
Summer, enhanced soft release, unconditioned	27 July 2018–27 May 2019	20	9	119.3 ± 2.9
Summer, enhanced soft release, conditioned	27 July 2018–27 May 2019	18	2	111.5 ± 2.2

effect of release season (fall or summer), release type (standard soft release or enhanced soft release), and conditioning (unconditioned or conditioned) on time until first movement, site fidelity, and survival. We predicted that time until first movement and site fidelity would be affected by release type, with enhanced cobble-bed soft releases leading to longer time until first movement and higher site fidelity. For survival, we predicted that release season and conditioning would both have an effect and individuals released in summer and reared in high-flow conditions would have the highest probability of postrelease survival.

MATERIALS AND METHODS

Study Area and Site Selection

The Blue River is the only river in Indiana, USA, with an extant population of Eastern Hellbenders. It originates in Washington County, Indiana, and flows south through Harrison and Crawford counties until its confluence with the Ohio River in Crawford County. We used a single study site approximately 500 m long that consists of a complex of alternating riffle, run, and pool habitats. The site has abundant cobble and gravel substrate with large, flat limestone boulders and bedrock crevices for shelter and a high level of hyporheic flow (Hillis and Bellis 1971; Burgmeier et al. 2011a). This site was used in a previous study as a trial release site for captive-reared Eastern Hellbenders (Kraus et al. 2017) and was chosen primarily because of its existing population of Eastern Hellbenders, large amount of contiguous high-quality habitat (approximately 500 m) compared to other potential study sites, and relative remoteness.

Captive Care and Environmental Enrichment

All Eastern Hellbenders in this study were from the same egg clutch collected from the Blue River in Fall 2013. All Eastern Hellbenders were unsexed and raised at Purdue University's Aquaculture Research Laboratory (ARL) in West Lafayette, Indiana. All individuals were reared in 132-L aquarium tanks for 2 yr and were then moved to 504-L polyethylene raceways (Pentair Filtration sump ModelS207095, Pentair Aquatic Ecosystems). As part of a separate laboratory study including these Eastern Hellbenders, 60 treatment individuals (referred to as conditioned) were raised in conditions with 0.2–0.3 m/s flow velocity, measured at the thalweg at midstream depth, similar to natural streamflow conditions. The 60 control individuals (referred to as unconditioned) were raised with a flow no greater than 0.05 m/s. For a detailed description of captive-rearing conditions and environmental enrichment, see Kenison and Williams (2018a).

Treatment Groups

Animals were released in either Fall 2017 or Summer 2018, making them 4-yr-old juveniles at the time of release. Four of the treatment groups were released in Fall 2017 and included unconditioned ($n = 20$) and conditioned ($n = 20$) individuals released using standard soft-release techniques and unconditioned ($n = 20$) and conditioned ($n = 20$) individuals released using enhanced soft-release techniques. Two of the treatment groups were released in Summer 2018 and included unconditioned ($n = 20$) and conditioned ($n = 18$) individuals released using enhanced soft-release techniques. There was substantial size variation between individuals in the cohort, and larger Eastern Hellbenders were preferentially chosen for Fall 2017 releases to ensure implanted transmitters were 5% of their total mass. Because of this preferential selection, the mean mass of individuals released in Summer 2018 was smaller than the fall cohorts though there was overlap among groups (Table 1).

Surgery

All individuals underwent surgical implantation of radio transmitters prior to release. We conducted surgeries on 80 Eastern Hellbenders on 2 October 2017 and 3 October 2017 and 39 Eastern Hellbenders on 11 June 2018 at ARL. One individual from the 2018 group experienced complications related to surgery and was not released as part of the study. All surgeries were conducted by licensed veterinarians or by an individual trained by Purdue University's Laboratory Animal Program veterinarians. All 2017 Eastern Hellbenders were implanted with a 5.2-g SB-2 model transmitter with a wrapped whip antenna (Holohil Systems, Ltd.), and all 2018 Eastern Hellbenders were implanted with a 4.0-g Model F1170 transmitter with an internal coil antenna (Advanced Telemetry Systems, Inc.). All Eastern Hellbenders were implanted with a 134.2-kHz International Standards Organization passive integrated transponder tag (12.5 mm long; Biomark, Inc.).

All 2017 surgeries followed the surgical methods described in Stouffer et al. (1983). All 2017 individuals were held for 3 d postsurgery in temporary recovery tanks before being moved back to their assigned raceways until release. We modified our protocol for 2018 surgeries by adopting a paramedial incision (rather than transverse) and increasing postsurgical recovery time from 3 to 30 d. This increased recovery time was required to accommodate several individuals that needed additional sutures after they experienced postsurgical dehiscence shortly after surgery.

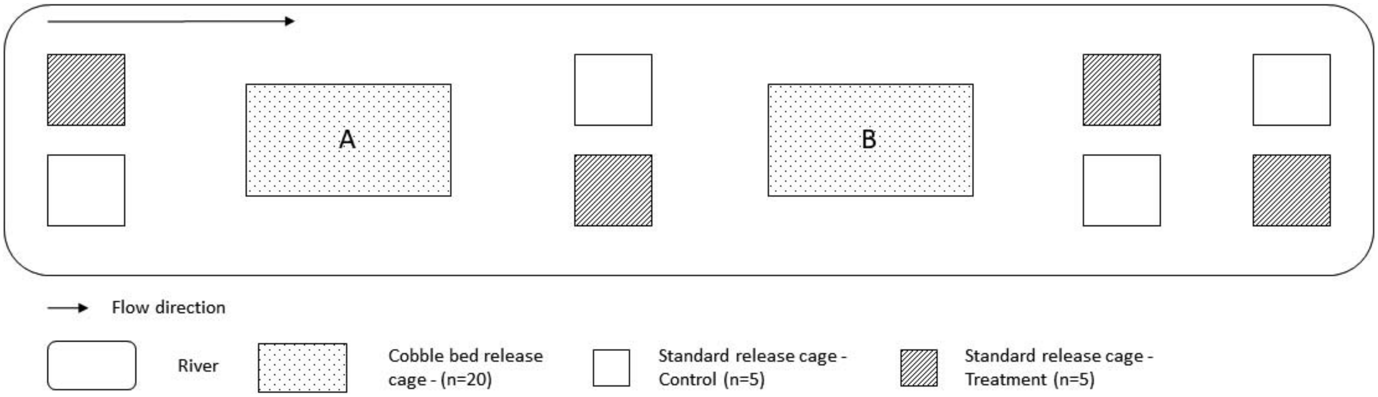

FIG. 1.—Release design (not to scale) of different soft-release types installed throughout a 200-m section of the Blue River, Indiana, for Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) released in Fall 2017 and Summer 2018. Each cobble bed received 20 individuals (*n* ¼ 20) and each standard release cage received 5 individuals (*n* ¼ 5).

Soft-release Structures

Two types of soft-release structures were used for this project: standalone mesh cages with shelter rocks (i.e., standard soft release) and permanent cobble beds with removable mesh cages (i.e., enhanced soft release). Cobble beds were 163 cm long by 76 cm wide by 23 cm tall. They were comprised of a 9-gauge woven wire cattle fence frame surrounded by 14-gauge, 2-in by 1-in (5.1 3 2.5-cm) steel fencing. These were placed on the stream bottom in the release site approximately 1 mo prior to release and filled with 6-in (15-cm) limestone riprap, though the actual size of included stones typically ranged from approximately 10–26 cm. Two cobble beds were placed at the upstream and downstream end of the release site (hereafter cobble Bed A and cobble Bed B, respectively).

The removable mesh cages for placement over cobble beds were 213 cm long by 91 cm wide by 30 cm high. They were comprised of a 1-in polyvinyl chloride pipe frame surrounded on five sides by ¼-in (0.64-cm) square seine netting zip-tied to the frame. An approximately 20-cm skirt of extra mesh fabric extended around the entire bottom of the cage. A large zipper was installed in the top center of the cage and two smaller zippers were installed on top at each end to allow for access to different parts of the cage. These cages were placed over the cobble beds on the day of release and secured with additional riprap on the skirt.

The standard soft-release cages were constructed in the same manner as the removable mesh cages but were 122 cm long by 122 cm wide by 46 cm tall. A single large zipper was placed in the top of each cage. On the day of the release, three approximately 51-cm-diameter flat slab rocks were positioned on the river bottom and a standard release cage was placed over top. The cages were secured with additional riprap on the skirt.

Field Methods

We released 80 Eastern Hellbenders between 1 November 2017 and 2 November 2017. We released 20 conditioned and 20 unconditioned individuals each day into either cobble beds or standard release cages installed throughout a 200-m section of our release site (Fig. 1). We released 10 conditioned and 10 unconditioned individuals into each of two large, permanent cobble beds surrounded by a temporary mesh cage, approximately 140 m apart. We released the remaining 20 conditioned and 20 unconditioned individuals into four pairs of standard release cages (eight total cages). Each cage per pair received five unconditioned Eastern Hellbenders or five conditioned Eastern Hellbenders (Fig. 1). Standard release cages received only five Eastern Hellbenders each because of the much lower volume of habitat within each cage compared with the cobble beds.

The temporary mesh cages and the standard release cages were scheduled to be removed on 6 November 2017. However, flooding on 6 November 2017 prevented us from accessing the cages. We removed the temporary mesh cages from the cobble beds and also three standard release cages on 14 November 2017. The five remaining standard soft-release cages had been dislodged and displaced downstream during the flooding. All Eastern Hellbenders received at least four full days of soft release.

We released 38 Eastern Hellbenders, 18 conditioned and 20 unconditioned, on 27 July 2018. We released 9 conditioned and 10 unconditioned Eastern Hellbenders into each of two large, permanent cobble beds (approximately 140 m apart) surrounded by a temporary mesh cage. The temporary mesh cages were removed on 31 July 2018.

We radio-tracked all Eastern Hellbenders one to three times weekly, weather permitting, from November 2017 through September 2018 (2017 fall release) and July 2018 through August 2019 (2018 summer release). We used a three-element Yagi antenna with a TRX-2000WR telemetry receiver (Wildlife Materials, Inc.). When we located individuals, we manually recorded whether an individual had moved from its previous location and marked a Universal Transverse Mercator (Zone 16N) coordinate, with at least 4 m accuracy, using a Garmin GPSMap 64st global positioning system unit (Garmin Ltd.). Survival of individuals was verified intermittently throughout the study but only for individuals that could be extracted from under shelter rocks without lifting the rock so as not to alter the habitat and increase the probability of shelter or site abandonment.

Statistical Analysis

We examined three dependent variables to determine whether there were differences between treatment groups (Table 2): days until first movement, site retention, and

TABLE 2.—Nested model structure and results of likelihood ratio significance tests for three models describing movement and survival of captive-reared Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) released in the Blue River, Indiana, in 2017 and 2018. We report degrees of freedom (df), likelihood ratio (LR), and significance level (*P*). Independent variables were only retained for the final model if their addition improved model fit ($\alpha = 0.05$).

	Dependent variable								
	Days until first movement			Site retention			Survival		
Model structure	df	LR	*P*	df	LR	*P*	df	LR	*P*
Intercept only	116			116			116		
Treatment	111	79.88	< 0.01	111	10.68	0.05	111	14.19	0.01
Treatment + mass	110	0.16	0.69	110	< 0.01	0.98	110	0.93	0.33
Treatment + mass + days until first movement				109	0.02	0.89	109	0.03	0.86

survival. We defined days until first movement as the number of days from the release of Eastern Hellbenders into soft-release cages until the last day an individual was found in the soft-release cage before being subsequently found in a new location away from the cage. We used the date of release rather than the date the cages were removed because we could not be sure when the high-water event dislodged the temporary standard soft-release cages, and because some individuals escaped the mesh cages in all treatment groups. Site retention was defined as the last known location of an individual, either inside or outside of the approximately 500-m release site, during a 10-mo (304-d) tracking period. Although the earliest signal loss for Eastern Hellbenders in both releases was 264 d, most transmitters were active past 264 d. We chose 304 d (10 mo) as a comparison period because most of the known deaths occurred in a 17-d window between 7 May and 24 May (71% of the fall cohort deaths and 55% of the spring cohort deaths), and we wanted to ensure that this high-risk time was included in the analysis for both groups. Only three known deaths occurred past the 304-d comparison period.

We conducted all analyses in R (v3.6.2; R Core Team 2019). We developed generalized linear models to test for treatment group differences for days until first movement and site retention. We used a negative binomial generalized linear model to model the number of days until first movement, because the full Poisson model demonstrated considerable overdispersion (c-hat = 31.6). We used logistic regression to model site retention. If individuals were in the release site on the last day they were observed or right-censored at 304 d, they were coded as a 1. We used a Cox proportional-hazards model to test for differences in survival time (Cox 1972). Individuals were coded as mortalities on the first day they were found dead. Eastern Hellbenders were right-censored retroactively on the last day their signal was detected if they were never detected again or at 304 d if they were still present in the study area. Individuals were assumed alive if they were located under suitable instream shelter, had recently moved, or had recently been visually verified as alive. We used the coxph function (Therneau and Lumely 2014) to fit the survival models and the survfit function (Therneau and Lumely 2014) to obtain Kaplain–Meier estimates of 10-mo survivorship in treatment groups. Because we were able to track the Summer 2018 release for an extended period of time, we also obtained Kaplan–Meier survival estimates for the two summer treatment groups at 365 d.

To fit final models, we developed a series of nested candidate models based on our predictions of independent variable importance, performed likelihood-ratio tests with $\alpha = 0.05$, and fitted a final best model to each dependent variable (Table 2). Because of the high variation in animal mass at release, our most saturated model for all dependent variables included treatment group and mass at release. In addition, for site retention and survival, the most saturated models also included days until first movement in case initial movement patterns affected site retention or survival rates. The saturated models were compared to models with just treatment groups as covariates, which were compared to intercept-only null models. We retained the independent variables in the final model only if their addition improved model fit. The independent variables mass and days until first movement were centered and scaled prior to entry in the model. Treatment groups were coded as dummy variables with the fall, standard soft release, unconditioned group acting as the baseline. This coding allowed for a natural comparison between the standard release conditions for captive-reared Eastern Hellbenders in Indiana and more advanced techniques. We inspected final model residuals to assess model assumptions.

RESULTS

We monitored 79 juvenile Eastern Hellbenders released in Fall 2017 for 304 d. One of the fall unconditioned Eastern Hellbenders was removed from the analysis because it was never detected postrelease. At the end of the study, 7 fall Eastern Hellbenders were alive, 34 were known dead, and 38 had unknown fates. Eastern Hellbenders released in Fall 2017 were right-censored between 14 and 302 d ($\bar{X} = 234.5 \pm 12.2$ d SE). We recovered only six bodies of Eastern Hellbenders, including one within the first 30 d that was alive but experiencing severe suture dehiscence. The cause of death could not be determined for most individuals. In 19 instances, transmitters were found one or more meters from the water line despite having no recent high-water events.

We monitored 38 juvenile Eastern Hellbenders released in Summer 2018 for 365 d. We monitored the summer cohort for longer than the fall cohort because of longer transmitter life. At Day 304, 20 Eastern Hellbenders were alive, 11 were known dead, and 7 had unknown fates. Eastern Hellbenders released in the summer of 2018 were right-censored between 90 and 297 d ($\bar{X} = 236.6 \pm 26.5$ d SE). At the end of the study (365 d), 15 Eastern Hellbenders were alive, 13 were dead, and 10 had unknown fates. We recovered only four bodies, and in eight instances transmitters were found one or more meters from the water line despite there being no high-water events since the date of last location. The remaining five transmitters were found in the water or near the waterline.

TABLE 3.—Final results for three models describing the movement and survival of captive-reared Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) released in the Blue River, Indiana, in 2017 and 2018. For each model covariate, we report coefficient estimates and associated standard errors (SE) and significance levels (*P*). All treatment group estimates are relative to the baseline group of unconditioned individuals released in Fall 2017 using standard soft release techniques.

	Dependent variable								
	Days until frst movement			Site retention			Survival		
Covariates	Estimate	SE	*P*	Estimate	SE	*P*	Estimate	SE	*P*
Intercept	1.83	0.27	¸ 0.01	1.73	0.63	¸ 0.01	—	—	—
Fall, standard soft release, conditioned	0.36	0.38	0.33	0.35	0.84	0.68	0.52	0.49	0.72
Fall, enhanced soft release, unconditioned	2.93	0.38	¸ 0.01	1.63	0.78	0.04	0.20	0.55	0.28
Fall, enhanced soft release, conditioned	3.07	0.37	¸ 0.01	1.53	0.77	0.05	0.20	0.49	0.68
Summer, enhanced soft release, unconditioned	1.53	0.37	¸ 0.01	1.73	0.77	0.02	0.25	0.51	0.62
Summer, enhanced soft release, conditioned	1.04	0.38	¸ 0.01	1.51	0.79	0.05	1.76	0.81	0.03
$\hat{c}$[a]	0.77	0.11	—	—	—	—	—	—	—

[a] The overdispersion parameter.

For all the dependent variables, the only covariate to significantly improve model fits was treatment (Table 2). In the model for days until first movement, the coefficients for the fall enhanced soft-release groups (conditioned and unconditioned) and the summer enhanced soft-release groups (conditioned and unconditioned) were significantly different than the fall standard soft-release unconditioned group (Table 3). In the fall groups, enhanced soft release delayed the time until first movement compared to the standard soft release (Table 3, Fig. 2). That is, days until first movement for the standard soft-release groups was 6.3 d (95% confidence interval [CI] ¼ 1.2–8.6) for the unconditioned group and 9.0 d (95% CI ¼ 1.3–11.5) for the conditioned group. In contrast, days until first movement for the enhanced soft-release groups was 117.3 d (95% CI ¼ 16.6–149.9) for the unconditioned group and 134.2 d (95% CI ¼ 12.1–157.9) for the conditioned group. In the summer groups, enhanced soft release also delayed the time until first movement compared to the fall standard soft release, although the magnitude of the effect of was smaller (Fig. 2). That is, days to first movement was 28.9 d (95% CI ¼ 11.1–50.6) for the unconditioned group and 17.7 d (95% CI ¼ 5.1–27.8) for the conditioned group.

Enhanced soft releases more than doubled the probability of site retention (Table 3). The overall probability of site retention was 0.46 (95% CI ¼ 0.36–0.58) in the enhanced soft release groups (Fig. 3). It was significantly lower in the unconditioned (0.15; 95% CI ¼ 0–0.31) and conditioned (0.20; 95% CI ¼ 0.02–0.38) standard soft release groups.

The only treatment group to demonstrate a statistically significant difference in survival probability from the baseline group was the summer, enhanced soft-release, conditioned group (Table 3). The probability of survival for their cohort over 10 mo (304 d) was the highest of any of the groups at 0.88 (95% CI ¼ 0.73–1.0), and was nearly double that of the fall, standard soft-release, unconditioned group at 0.47 (95% CI ¼ 0.26–0.84) (Fig. 4). At 365 d, survivorship in the summer conditioned group was still higher at 0.74 (95% CI ¼ 0.55–0.99) than the summer unconditioned group at 0.50 (95% CI ¼ 0.31–0.79).

DISCUSSION

This study is the first to combine environmental enrichment and seasonal releases with enhanced soft-release techniques and radiotelemetry to evaluate combined effects on movement, site retention, and survival for captive-reared

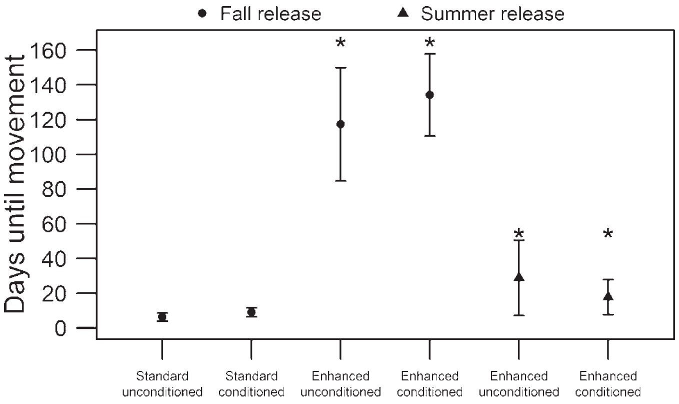

FIG. 2.—Mean number of days until first movement (with 95% confidence interval) for head-started Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) released in the Blue River, Indiana. Treatments were release season (fall or summer), release type (standard soft release or enhanced soft release), and conditioning status (unconditioned or conditioned). Asterisk (*) indicates a statistically significant (*α* ¼ 0.05) difference from the baseline group (fall standard soft release, unconditioned).

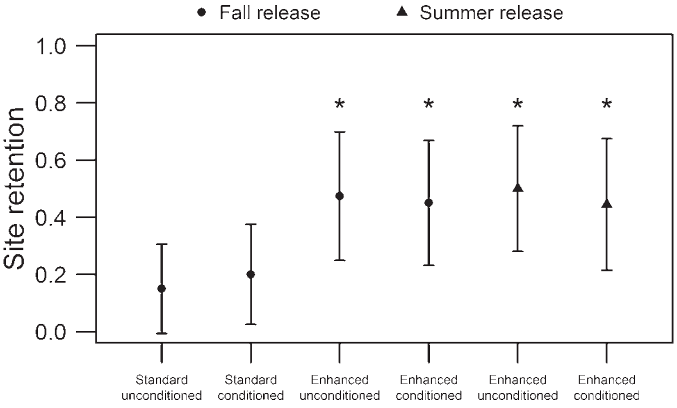

FIG. 3.—Site retention estimates (with 95% confidence interval) for six treatment groups of head-started Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) released in the Blue River, Indiana. Treatments were release season (fall or summer), release type (standard soft release or enhanced soft release), and conditioning status (unconditioned or conditioned). Asterisk (*) indicates a statistically significant (*α* ¼ 0.05) difference from the baseline group (fall standard soft release, unconditioned).

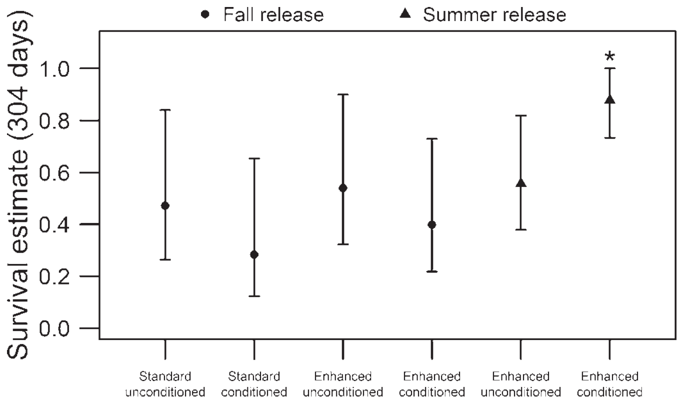

FIG. 4.—Ten-month survivorship estimates (with 95% confidence interval) for six treatment groups of head-started Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) released in the Blue River, Indiana. Treatments were release season (fall or summer), release type (standard soft release or enhanced soft release), and conditioning status (unconditioned or conditioned). Asterisk (*) indicates a statistically significant (*a* ¼ 0.05) difference from the baseline group (fall standard soft release, unconditioned).

aquatic salamanders being released into the wild. As expected, we found that treatment groups released using enhanced soft-release techniques had a higher site retention. Unexpectedly, we found that flow conditioning had a positive effect on survivorship in the summer cohort, but there was no corresponding increase in survival in the conditioned fall cohorts. Based on our results, we recommend using a combination of advanced techniques to improve ultimate conservation outcomes and decrease costs to captive-rearing programs over time.

Enhanced soft releases into cobble beds were highly effective at delaying the time until first movement when compared with standard soft releases. This delay to movement could give released Eastern Hellbenders additional opportunities to acclimate to the novel conditions experienced in the river. Enhanced soft releases also proved more effective than standard soft releases at retaining individuals within the area of the release site. Site retention is especially important for Eastern Hellbenders in Indiana since habitat in the Blue River tends to be isolated to short stretches of river and separated by large patches of relatively suboptimal areas (Burgmeier et al. 2011b). There was no direct effect of days until first movement on an individual's probability of site retention, indicating the cobble bed installations had effects beyond the initial postrelease period. A high-water event dislodged five of the fall standard soft-release cages sometime between 4 d and the removal of the other cages. It is possible that this artificially increased the time until first movement for some individuals; however, the maximum time until first movement in the remaining secured standard soft-release cages did not exceed 15 d and there was no direct effect of days until first movement on site retention, so we do not expect this dramatically affected our end points of retention or survival. Eastern Hellbenders from all treatment groups returned to use the augmented habitat throughout the study, which is unsurprising given the documented preference for coarse substrates in juvenile age classes (Bodinof et al. 2012c; Hecht et al. 2019; Unger et al. 2020b). Cobble substrates provide cover for the secretive species and sites to forage for macroinvertebrates (Duan et al. 2008). Importantly, the cobble beds were also stable over the course of the study and withstood several high-volume flood events.

The benefits of delayed dispersal and site retention appear to depend on the timing of release. Our fall release was conducted in early November, a time when Eastern Hellbenders in Indiana sharply decrease their movement (Burgmeier et al. 2011a). In the fall cohort, 76% of individuals in the enhanced soft-release group stayed in place for at least 3 mo indicating they chose to overwinter in the cobble beds instead of immediately exploring the surrounding habitat. Translocated Hellbenders released in the spring and summer demonstrate a pattern of high levels of initial movement followed by decreasing home range size over time (Bodinof et al. 2012a; McCallen et al. 2018). This study provides further evidence that captive-reared Hellbenders released in the fall show the reverse pattern and delay initial exploratory movements until later seasons (Bodinof et al. 2012a). This delay in exploratory behavior may leave fall-released cohorts vulnerable to predation and unable to provision adequate food resources (Le Gouar et al. 2012) during a particularly important foraging period (Nickerson and Mays 1973). Conversely, the enhanced soft-release groups that were released in the summer spent considerably less time initially in the cobble beds than the enhanced soft-release groups released in the fall (though time until first movement was still significantly greater in the summer-released group compared to the baseline). While many individuals started exploring the environment quickly upon release, 47% of individuals in the enhanced soft-release cohort that was released in the summer spent at least 19 d in the cobble beds. For these individuals, leaving the cobble beds coincided with an uptick of movement that occurs when males start establishing breeding territories (Burgmeier et al. 2011a; McCallen et al. 2018). Captive-reared Hellbenders often disperse during the first 20 d postrelease (Bodinof et al. 2012a), so this delayed time until movement may still have contributed to improved outcomes in the summer cohort.

Although days until first movement and site retention were increased in all enhanced soft-release treatment groups, survival was significantly higher only in the conditioned group released in the summer. We studied the behavior and morphology of these individuals when they were in the laboratory, prior to release, and found that conditioning resulted in a significant improvement in swim performance and morphological features favorable to inhabiting a lotic environment (Kenison and Williams 2018a). Thus, we hypothesized that after release into the field, conditioned individuals would more successfully navigate the lotic environment than unconditioned individuals, leading to increased survival rates. The reason we did not see increased survival in conditioned individuals released in the fall may be related to the nature of seasonal threats in the two cohorts. In both groups, most known mortalities occurred in May when movement rates of Eastern Hellbenders are known to increase due to foraging (Humphries and Pauley 2000; Humphries 2007; Burgmeier et al. 2011b). During May 2019, there was one high-flow flood event in the Blue River, and high-flow conditioning may have conferred a direct advantage to captive-reared Eastern Hellbenders during flooding through improved swim performance (Kenison and Williams 2018a; Franssen et al. 2021). Alternatively, in May 2018 the river never flooded and high-flow conditions were not a threat to the fall-released, captive-reared cohort during seasonal movement activities. In conditions of unseasonably low flow, disease or predators are more likely sources of mortality and flow conditioning would confer no direct advantage to the fall cohort.

An alternative hypothesis for the mechanism influencing survival differences in the conditioned and unconditioned captive-reared Hellbenders is the indirect effects of stress (Dickens et al. 2010). The interaction of stress and release type has received little empirical attention but warrants further consideration (Teixeira et al. 2007). If conditioned Hellbenders experience a decreased acute or chronic stress response upon release because they are already acclimated to high flow conditions (Dickens et al. 2010), then it may increase their probability of survival. Prolonged elevation of stress hormones in animals can cause impaired cognitive function (de Quervain et al. 1998; Mendl 1999) which can affect their ability to obtain resources and increase the likelihood of predation (Teixeira et al. 2007). It may also suppress immune system function (Romero and Butler 2007), which can decrease the probability of survival when individuals are challenged by pathogens or parasites (Dhabhar et al. 1996; Bortolotti et al. 2009; Hing et al. 2017). The relatively longer amount of time that the summer cohort spent undergoing the conditioning treatment (eight additional months) may have also increased their chances of survival relative to the fall cohort, either through additional changes in morphology and swim performance or increased acclimation to the high flow conditions. More research is needed to explore the mechanisms driving differential survival in conditioned and unconditioned groups and determine the optimal amount of conditioning needed to efficiently reach captive-rearing program recovery goals for Hellbenders.

Predation is suspected as a major cause of mortality in this study. One individual was positively confirmed to have been eaten by a Common Watersnake (*Nerodia sipedon*) and a second was confirmed to have been eaten by a Common Snapping Turtle (*Chelydra serpentina*). We saw no direct evidence of mammal predation; however, Raccoons (*Procyon lotor*; Lipps 2013) and River Otters (*Lontra canadensis*; Hecht et al. 2014) are well-documented predators of Hellbenders. We suspect that mammalian predation was a major cause of mortality in this study based on discovering several transmitters located greater than 1 m above the water line with no recent high-water events. However, isolated transmitters discovered on banks could also be evidence of mammalian scavenging behavior. High predation rates (Beck et al. 1991; Moseby et al. 2011; Berger-Tal et al. 2020), inadequate foraging (Bright and Morris 1994; Mathews et al. 2005; Jule et al. 2008), and disease (Williams et al. 1988; Stockwell et al. 2008; Berger-Tal et al. 2020) are all commonly cited causes of mortality in translocations and none can be ruled out as factors in this study. Applying additional conditioning techniques for predator avoidance (Kenison and Williams 2018b; Tetzlaff et al. 2019a; Šmejkal et al. 2021) and immune defenses (Merrifeld et al. 2010; Kenison et al. 2020) could be critical for further improving the success of future captive-reared releases of Hellbenders.

When used simultaneously, summer releases, enhanced soft releases, and conditioning improved the postrelease survivorship of captive-reared Eastern Hellbenders. The annual survivorship estimate of the conditioned summer cohort was higher than previous captive-reared cohorts released at the same site in Indiana (Kraus et al. 2017) and was comparable to the previous highest reported survival rate for captive-reared Hellbenders (Bodinof et al. 2012b). Based on our results, we recommend the use of these advanced captive-rearing techniques. Flow conditioning can be implemented easily and relatively inexpensively using raceway systems (Kenison and Williams 2018a) rather than standard aquaria. Based on the seasonal differences we saw in survivorship gains, we recommend combining flow conditioning with releasing Hellbenders in late spring or early summer to ensure individuals have adequate time to explore their habitat and develop home ranges prior to dormancy. Finally, we recommend using cobble-bed soft releases, when feasible, to help delay dispersal and improve site retention. However, the placement of soft-release structures should be taken into consideration to avoid areas of especially high flow or those frequented or easily accessed by predators. Future work should focus on determining the optimal length of conditioning prerelease and whether different combinations of conditioning techniques (e.g., flow, predator, and microbiota) might have a synergistic effect on survivorship. Furthermore, soft-release techniques should continue to be evaluated to better understand the nuances of in-stream placement. Improving captive-rearing techniques and the subsequent survivorship of released Hellbenders could have significant positive effects on the success of future releases and the long-term status of the species in the wild.

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LITERATURE CITED

Beck, B.B., D.G. Kleiman, J.M. Dietz, I. Castro, C. Carvalho, A. Martins, and B. Rettberg-Beck. 1991. Losses and reproduction in reintroduced golden lion tamarins, *Leontopithecus rosalia*. Dodo: Journal of Jersey Wildlife Preservation Trust 27:50–61.

Berger-Tal, O., D.T. Blumstein, and R.R. Swaisgood. 2020. Conservation translocations: A review of common difficulties and promising directions. Animal Conservation 23:121–131.

Biggins, E., J.L. Godbey, L.R. Hanebury, B. Luce, P.E. Marinari, M.R. Matchett, and A. Vargas. 1998. The effect of rearing methods on survival of reintroduced black-footed ferrets. The Journal of Wildlife Management 62:643–653.

Blais, D.P. 1989. Movement, Home Range, and Other Aspects of the Biology of the Eastern Hellbender (*Cryptobranchus alleganiensis*

alleganiensis): A Radio Telemetric Study. Master's thesis, State University of New York at Binghamton, USA.

Blythe, R.M., T.J. Smyser, S.A. Johnson, and R.K. Swihart. 2015. Post-release survival of captive-reared Allegheny woodrats. Animal Conservation 18:186–195.

Bodinof, C.M., J.T. Briggler, R.E. Junge, J. Beringer, M.D. Wanner, C.D. Schuette, J. Ettling, R.A. Gitzen, and J.J. Millspaugh. 2012a. Postrelease movements of captive-reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*). Herpetologica 68:160–173.

Bodinof, C.M., J.T. Briggler, R.E. Junge, T. Mong, J. Beringer, M.D. Wanner, C.D. Schuette, J. Ettling, and J.J. Millspaugh. 2012b. Survival and body condition of captive-reared juvenile Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) following translocation to the wild. Copeia 2012:150–159.

Bodinof, C.M., J.T. Briggler, R.E. Junge, T. Mong, J. Beringer, M.D. Wanner, C.D. Schuette, J. Ettling, and J.J. Millspaugh. 2012c. Habitat attributes associated with short-term settlement of Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) salamanders following translocation in the wild. Freshwater Biology 57:178–192.

Boerner, J. 2014. Comparison of Movement Patterns in Captive-released Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) using Three Different Release Methods. Master's thesis, State University of New York Buffalo State College, USA.

Bortolotti, G.R., F. Mougeot, J. Martinez-Padilla, L.M.I. Webster, and S.B. Piertney. 2009. Physiological stress mediates the honesty of social signals. PLOS ONE 4:e4983.

Brichieri-Colombi, T.A., and A. Moehrenschlager. 2016. Alignment of threat, effort, and perceived success in North American conservation translocations. Conservation Biology 30:1159–1172.

Bright, P.W., and P.A. Morris. 1994. Animal translocation for conservation: Performance of dormice in relation to release methods, origin, and season. Journal of Applied Ecology 31:699–708.

Burgmeier, N.G., T.M. Sutton, and R.N. Williams. 2011a. Spatial ecology of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Herpetologica 67:135–145.

Burgmeier, N.G., S.D. Unger, T.M. Sutton, and R.N. Williams. 2011b. Population status of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Herpetologica 67:135–145.

Cox, D.R. 1972. Regression models and life tables. Journal of the Royal Statistical Society 34:187–220.

Cresswell, R.C., and R. Williams. 1983. Post-stocking movements and recapture of hatchery-reared trout released into flowing waters—Effect of prior acclimation to flow. Journal of Fish Biology 23:265–276.

de Quervain, D.F., B. Roozendaal, and J. McGaugh. 1998. Stress and glucocorticoids impair retrieval of long-term spatial memory. Nature 394:787–790.

Dhabhar, F.S., A.H. Miller, B.S. McEwen, and R.L. Spencer. 1996. Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. The Journal of Immunology 157:1638–1644.

Dickens, M.J., D.J. Delehanty, and L.M. Romero. 2010. Stress: An inevitable component of animal translocation. Biological Conservation 143:1329–1341.

Dodd, C.K., and R.A. Seigel. 1991. Relocation, repatriation, and translocation of amphibians and reptiles: Are they conservation strategies that work? Herpetologica 47:336–350.

Dodd, C.K., and R.A. Seigel. 2002. Translocations of amphibians: Proven management method of experimental technique? Conservation Biology 16:552–554.

Duan, X., Z. Wang, and S. Tian. 2008. Effect of streambed substrate on macroinvertebrate biodiversity. Frontiers of Environmental Science & Engineering in China 2:122–128.

Fischer, J., and D.B. Lindenmayer. 2000. An assessment of the published results of animal relocations. Biological Conservation 96:1–11.

Franssen, N.R., S.L. Durst, E.I. Gilbert, W.K. Knight, and M. Ulibarri. 2021. Flow conditioning of hatchery-reared razorback sucker increases apparent survival in the wild. North American Journal of Fisheries Management 41:545–555.

Germano, J.M., and P.J. Bishop. 2009. Suitability of amphibians and reptiles for translocation. Conservation Biology 23:7–15.

Griffith, B., J.M. Scott, J.W. Carpenter, and C. Reed. 1989. Translocation as a species conservation tool: Status and strategy. Science 245:477–480.

Hecht, K.A., M.A. Nickerson, and R. Vollbrecht. 2014. *Cryptobranchus alleganiensis* (Hellbender). Predation. Herpetological Review 45:471.

Hecht, K.A., M.J. Freake, M.A. Nickerson, and P. Colclough. 2019. Hellbender salamanders (*Cryptobranchus alleganiensis*) exhibit an ontogenetic shift in microhabitat use in a Blue Ridge physiographic region stream. Copeia 107:152–159.

Hernandez-Gomez, O., J.T. Briggler, and R.N. Williams. 2019. Captivity-induced changes in the skin microbial communities of Hellbenders (*Cryptobranchus alleganiensis*). Microbial Ecology 77:782–793.

Hester, J.M., S.J. Price, and M.E. Dorcas. 2008. Effects of relocation on movements and home ranges of Eastern Box Turtles. The Journal of Wildlife Management 72:772–777.

Hillis, R.E., and E.D. Bellis. 1971. Some aspects of the ecology of the Hellbender in a Pennsylvania stream. Journal of Herpetology 5:121–126.

Hing, S., A.S. Northover, E.J. Narayan, A.F Wayne, K.L. Jones, S. Keatley, R.C.A. Thompson, and S.S. Godfrey. 2017. Evaluating stress physiology and parasite infection parameters in the translocation of critically endangered woylies (*Bettongia penicillata*). EcoHealth 14:128–138.

Humphries, W.J. 2005. *Cryptobranchus alleganiensis* (hellbender). Displacement by a food. Herpetological Review 36:428.

Humphries, W.J. 2007. Diurnal seasonal activity of *Cryptobranchus alleganiensis* (Hellbender) in North Carolina. Southeastern Naturalist 6:135–140.

Humphries, W.J., and T.K. Pauley. 2000. Seasonal changes in nocturnal activity of the hellbender, *Cryptobranchus alleganiensis*, in West Virginia. Journal of Herpetology 34:604–607.

IDNR (Indiana Department of Natural Resources). 2017. State Wildlife Grant—Indiana: Assessing Hellbender Habitat for Potential Reintroductions. Available at https://www.in.gov/dnr/fsh-and-wildlife/files/fw-2017wsr_HellbenderHabitat.pdf. Accessed on 30 August 2022. Indiana Department of Natural Resources, USA.

Jachowski, D.S., J.J. Millspaugh, P.L. Angermeier, and R. Slotow (eds.). 2016. Reintroduction of Fish and Wildlife Populations. University of California Press, USA.

Jachowski, C.M.B., B.E. Ross, and W.A. Hopkins. 2020. Evaluating artificial shelter arrays as a minimally invasive monitoring tool for the hellbender *Cryptobranchus alleganiensis*. Endangered Species Research 41:167–181.

Jule, K.R., L.A. Leaver, and S.E. Lea. 2008. The effects of captive experience on reintroduction survival in carnivores: A review and analysis. Biological Conservation 141:355–363.

Kelly, J.L., A.E. Magurran, and C. Macias-Garcia. 2005. The influence of rearing experience on the behaviour of an endangered Mexican fish, *Skiffa multipunctata*. Biological Conservation 122:223–230.

Kenison, E.K., and R.N. Williams. 2018a. Rearing captive eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) with moving water improves swim performance. Applied Animal Behaviour Science 202:112–118.

Kenison, E.K., and R.N. Williams. 2018b. Training for translocation: Predator conditioning behavioral plasticity and physiological changes in captive eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*)(Cryptobranchidae, Amphibia). Diversity 10:13. DOI: https://dx.doi.org/10.3390/d10010013

Kenison, E.K., O. Herna´ndez-Go´ mez, and R.N. Williams. 2020. A novel bioaugmentation technique effectively increases the skin-associated microbial diversity of captive eastern hellbenders. Conservation Physiology 8:coaa040. DOI: https://dx.doi.org/10.1093/conphys/coaa040

Knox, C.D., and J.M. Monks. 2014. Penning prior to release decreases post-translocation dispersal of jeweled geckos. Animal Conservation 17:18–26.

Kraus, B.T., E.B. McCallen, and R.N. Williams. 2017. Evaluating the survival of translocated adult and captive-reared, juvenile Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*). Herpetologica 73:271–276.

Le Gouar, P., J.B. Mihoub, and F. Sarrazin. 2012. Dispersal and habitat selection: Behavioural and spatial constraints for animal translocations. Pp. 138–164 in Reintroduction Biology: Integrating Science and Management (J.G. Ewen, D.P. Armstrong, K.A. Parker, and P.J. Seddon, eds.). Wiley-Blackwell, UK.

Lipps, G.J., Jr. 2013. Eastern Hellbender *Cryptobranchus alleganiensis*. Pp. 187–208 in Amphibians of Ohio (R.A. Pfingsten, J.G. Davis, T.O. Matson, G.J. Lipps, Jr., D. Wynn, and B.J. Armitage, eds.). Ohio Biological Survey, USA.

Mathews, F., M. Orros, G. McLaren, M. Gelling, and R. Foster. 2005. Keeping fit on the ark: Assessing the suitability of captive-bred animals for release. Biological Conservation 121:569–577.

Matthews, K.R. 2003. Response of Mountain Yellow-Legged Frogs, *Rana muscosa*, to short distance translocation. Journal of Herpetology 37:621–626.

Mayasich, J., D. Grandmaison, and C. Phillips. 2003. Eastern Hellbender status assessment report. Technical Report. Natural Resources Research Institute, USA.

Maynard, D.J., T.A. Flagg, C.V.W. Mahnken, and S.L. Schroder. 1996. Natural rearing technologies for increasing postrelease survival of hatchery-reared salmon. Bulletin of National Research Institute of Aquaculture, Supplement 2:71–77.

McCallen, E.B., B.T. Kraus, N.G. Burgmeier, S. Fei, and R.N. Williams. 2018. Movement and habitat use of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) following population augmentation. Herpetologica 74:283–293.

Mendl, M. 1999. Performing under pressure: Stress and cognitive function. Applied Animal Behaviour Science 65:221–244.

Merrifeld, D.L., A. Dimitroglou, A. Foey, S.J. Davies, R.T.M. Baker, J. Bøgwald, M. Castex, and E. Ringø. 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302:1–18.

Miller, K.A., T.P. Bell, and J.M. Germano. 2014. Understanding publication bias in reintroduction biology by assessing translocation of New Zealand's herpetofauna. Conservation Biology 28:1045–1056.

Moseby, K.E., J. Read, D. Paton, P. Copely, B. Hill, and H. Crisp. 2011. Predation determines the outcome of 10 reintroduction attempts in arid South Australia. Biological Conservation 144:2863–2872.

Nickerson, M.A., and C.E. Mays. 1973. The Hellbenders: North American Giant Salamanders. Milwaukee Public Museum Press, USA.

Nickerson, M.A., A.L. Pitt, and M.D. Prysby. 2007. The effects of fooding on hellbender salamander, *Cryptobranchus alleganiensis* Daudin, 1803, populations. Salamandra 43:111–117.

R Core Team. 2019. R: A language and environment for statistical computing, Version 3.6.2. Available at http://www.R-project.org/. R Foundation for Statistical Computing, Austria.

Reading, R.P., B. Miller, and D. Shepherdson. 2013. The value of enrichment to reintroduction success. Zoo Biology 32:332–341.

Roe, J.H., M.R. Frank, S.E. Gibson, O. Attum, and B.A. Kingsbury. 2010. No place like home: An experimental comparison of reintroduction strategies using snakes. Journal of Applied Ecology 47:1253–1261.

Romero, M.L., and L.K. Butler. 2007. Endocrinology of stress. International Journal of Comparative Psychology 20:89–95.

Serfass, T.l., L.M. Rymon, and R.P. Brooks. 1990. Feeding relationships of river otters in northeastern Pennsylvania. Transactions of the Northeast Section of the Wildlife Society 47:43–53.

Šmejkal, M., D. Bartoň, P. P., PodhorecBlabolil, A.T. Souza, V. Stejskal, Y. Stepanyshyna, and S. Tapkir. 2021. Does fish conditioning in aquaculture increase survival success in the wild? A case study on a cyprinid fish. Sustainability 13:13936. DOI: https://dx.doi.org/10.3390/su132413936

Smith, B.G. 1907. The life history and habits of *Cryptobranchus alleganiensis*. Biological Bulletin 13:5–39.

Snyder, N.F.R., S.R. Derrickson, S.R. Beissinger, J.W. Wiley, T.B. Smith, W.D. Toone, and B. Miller. 1996. Limitations of captive breeding in endangered species recovery. Conservation Biology 10:338–348.

Sonn, J.M., R.M. Utz, and C.L. Richards-Zawacki. 2019. Effects of latitudinal, seasonal, and daily temperature variations on chytrid fungal infections in a North American frog. Ecosphere 10:e02892.

Soorae, P.S. (ed.). 2018. Global Reintroduction Perspectives: 2018. Case Studies from Around the Globe. Arafah Printing Press LLC, United Arab Emirates.

Stockwell, M., S. Clulow, J. Clulow, and M. Mahony. 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. Australian Zoologist 34:379–386.

Stoinski, T.S., B.B. Beck, M.A. Bloomsmith, and T.L. Maple. 2003. A behavioral comparison of captive-born, reintroduced golden lion tamarins and their wild-born offspring. Behavior 140:137–160.

Stouffer, R.H., Jr., J.E. Gates, C.H. Hocutt, and J.R. Stauffer, Jr. 1983. Radio-tracking endangered Hellbenders. Wildlife Society Bulletin 11:384.

Teixeira, C.P., C.S. De Azevedo, M. Mendl, C.F. Cipreste, and R.J. Young. 2007. Revisiting translocation and reintroduction programmes: The importance of considering stress. Animal Behaviour 73:1–13.

Tetzlaff, S.J., J.H. Sperry, and B.A. DeGregorio. 2019a. Effects of antipredator training, environmental enrichment, and soft release on wildlife translocations: A review and meta-analysis. Biological Conservation 236:324–331.

Tetzlaff, S.J., J.H. Sperry, and B.A. DeGregorio. 2019b. Tradeoffs with growth and behavior for captive box turtles head-started with environmental enrichment. Diversity 11:40. DOI: https://dx.doi.org/10.3390/d11030040

Therneau, T.M., and T. Lumley. 2014. R Package survival: Survival analysis. Available at https://www.rdocumentation.org/packages/survival/versions/3.3-1. Accessed on 28 March 2022. R Foundation for Statistical Computing, Austria.

Unger, S., C.M.B. Jachowski, L. Diaz, and L.A. Williams. 2020a. Shelter guarding behavior of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in North Carolina streams. Southeastern Naturalist 19:742–758.

Unger, S., C.M.B. Jachowski, L. Diaz, and L.A. Williams. 2020b. Observations on habitat preference of juvenile eastern hellbender salamanders (*Cryptobranchus alleganiensis*). Acta Ethologica 23:119–124.

Unger, S.D., L.A. Williams, and J.D. Groves. 2021. *Cryptobranchus alleganiensis alleganiensis* (Eastern Hellbender): Larval displacement due to fooding. Herpetological Review 52:598.

Wheeler, B.A., E. Prosen, A. Mathis, and R.F. Wilkinson. 2003. Population declines of a long-lived salamander: A 20þ year study of Hellbenders, *Cryptobranchus alleganiensis*. Biological Conservation 109:151–156.

Williams, E.S., E.T. Thorne, M.J.G. Appel, and D.W. Belitsky. 1988. Canine distemper in black footed ferrets (*Mustela nigripes*) from Wyoming. Journal of Wildlife Diseases 24:385–398.

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