Building Eastern Hellbender (Cryptobranchus a. alleganiensis) Populations through Reintroduction of Head-Started Individuals

Matthew D. Kaunert (✉ mk235207@ohio.edu )
Ohio University

Ryan K. Brown
Ohio University

Stephen Spear
United States Geological Survey

Peter B. Johantgen
Columbus Zoo and Aquarium

Viorel D. Popescu
Ohio University

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Abstract

Freshwater biodiversity is declining at a fast pace despite significant efforts directed towards the management and conservation of aquatic systems. Specifically, amphibians are among the most threatened taxa, with loss of aquatic habitat and alteration of habitat quality among important drivers of decline. Eastern Hellbenders are one of North America’s most iconic stream amphibian species, a sentinel of stream health, and are experiencing rapid population declines throughout most of their range. Common conservation strategies include headstarting (raising animals in captivity from wild eggs until 3–4 years old) and releasing them into streams. However, the success of this strategy for rebuilding hellbender populations and the most optimal release scenarios have largely been unassessed. In this study, we use a cohort of 205 headstart animals released in several Ohio watersheds in 2018 to evaluate the success of headstarting and reintroduction efforts over 3 years. Using PIT-tag surveys over 25 occasions between July 2018 and November 2021 and capture-recapture models, we found that 3-year apparent survival post-release was 0.162 ± 0.061, with lower survival in the first year (0.383 ± 0.058) and higher in years 2 (0.696 ± 0.086) and 3 (0.609 ± 0.154). We used demographic simulations integrating survival data from this study and from the literature to evaluate the number of releases and timing of releases required for building self-sustaining populations. We found that, given the low survival post-release, releasing cohorts of N = 100 individuals at fewer suitable sites several times (e.g., 3 releases, 2 or 3 years apart) would be a better strategy to achieve a high number of breeding adults compared to single release events across multiple sites. In addition, Eastern Hellbender headstarting programs using wild eggs are highly beneficial in producing more animals reaching adulthood (up to 7 times) compared to allowing eggs to develop in the wild. This study emphasizes the need to monitor the success of reintroduction programs, particularly for species with cryptic lifestyles. It also provides evidence that headstarting can be a viable strategy for rebuilding Eastern Hellbender populations, particularly if implemented in tandem with other management actions such as improving habitat and water quality and mitigating other threats.

Introduction

Lotic habitats are among the most threatened on Earth, due to such threats as land use change, introduction of non-native species, resource extraction, and climate change (Strayer and Dudgeon 2010). As such, many freshwater stream taxa are experiencing precipitous declines in response to anthropogenic disturbance (Dudgeon et al. 2006; Strayer 2006). Amphibians are experiencing widespread declines (Adams et al. 2013; Grant et al. 2019) and now represent the most imperiled class of vertebrates with 41% of species currently listed as threatened or near-threatened (IUCN 2022). While stream amphibians are often considered to be sentinels for freshwater habitat integrity and ecosystem health (Welsh and Ollivier 1998; Riley et al. 2005; Barrett et al. 2010; Calderon et al. 2019), 62% of amphibian species associated with lotic habitats are currently experiencing rapid declines (Stuart et al. 2004).

One potential conservation action for declining amphibian populations is translocation, which is defined as the human-mediated movement of living organisms from one area with release in another (Griffith et
Translocation has increasingly been employed across a wide variety of taxa (Seddon et al. 2007; Resende et al. 2020) and is a common conservation strategy for many threatened species (Fischer and Lindenmayer 2000; Armstrong and Seddon 2008). However, translocation is often considered to have low efficacy (Pérez et al. 2012) or a high cost to success ratio (Beauchamp et al. 2000; Taggart et al. 2016). Determining the efficacy of reintroduction efforts is further complicated given various definitions of short- or long-term success (Seddon 1999; Gusset et al. 2008; Teixeira et al. 2007; IUCN/SSC 2013) and many translocation studies altogether fail to report information regarding success/failure of such efforts (Resende et al. 2020). While translocation is popular among terrestrial taxa such as birds and mammals (Fischer and Lindenmayer 2000; Seddon et al. 2005; Resende et al. 2020), aquatic taxa and amphibians are relatively underrepresented groups in programs involving translocation (Germano and Bishop 2009; Resende et al. 2020).

Head-starting is a specific type of translocation in which individuals are reared in captivity during their most vulnerable stage (e.g., early life stages) prior to reintroduction in an effort to increase post-release survival and recruitment rates (Dodd and Seigel 1991; Dodd 2005). However, while head-starting may be a required application for certain declining species (Stuart et al. 2004), it may only serve as a temporary solution until underlying mechanisms of declines can be better understood (Zippel and Mendelson 2008). Despite the potential for head-starting to augment wild populations, the strategy often results in mixed success (Dodd and Seigel 1991) due to emigration away from release sites (Matthews 2003), unsuitable habitat at release sites (White and Pyke 2008), naivety of reintroduced individuals (Stamps and Swaisgood 2007; Kenison and Williams 2018a,b), and the persistence of threats that originally drove population declines (Rickard 2006). Furthermore, head-starting programs often lack systematic post-release monitoring to estimate survival and persistence (Fischer and Lindenmayer 2000; Germano and Bishop 2009). Survivorship of introduced animals is a critical parameter for establishing viable populations and evaluating overall success of head-starting efforts (White and Pyke 2008). Focused monitoring of survival of released animal cohorts (Seddon et al. 2007; Sutherland et al. 2010; IUCN/SSC 2013) coupled with population modelling (Seddon 1999) could inform the design of efficient reintroduction programs aimed at improving persistence of head-started populations. Furthermore, assessing the trade-offs between costs of head-starting against the success in establishing or augmenting wild populations is essential for determining optimal reintroduction strategies (Rout et al. 2005, Kissel et al. 2014; Resende et al. 2020).

The Eastern Hellbender (*Cryptobranchus a. alleganiensis*), is a long-lived (> 25 years; Taber et al. 1975), large-bodied (≤ 74 cm) stream-obligate salamander endemic to the eastern United States (Nickerson and Mays 1973). Hellbenders specialize on well-oxygenated stream habitats with an abundance of large cover rocks and crayfish (Nickerson and Mays 1973; Peterson et al. 1989). Hellbenders have recently undergone rapid population declines driven by chronically suppressed recruitment throughout much of their range (Williams et al. 1981; Trauth et al. 1992; Mayasich et al. 2003; Wheeler et al. 2003; Briggler et al. 2007b; Foster et al. 2009; Freake and De Perno 2017). As such, hellbenders are now threatened or endangered in most states where they historically occurred (Mayasich et al. 2003), and the midwestern...
United States has experienced drastic declines in recent decades (Burgmeier et al. 2011; Lipps et al. 2013; Smeenk et al. 2021) due to a variety of suspected factors including land use change (Bodinof Jachowski and Hopkins 2018), degraded water quality (Pitt et al. 2017), and siltation (Unger et al. 2021). Populations in Ohio have declined by ~ 80% since the 1980s (Pfingsten 1990; Lipps et al. 2013) and Eastern Hellbenders have been listed as state-endangered since 1990. To augment wild populations, extensive captive-rearing and reintroduction programs are now the primary method for hellbender recovery within Ohio (Lipps et al 2013; Smeenk et al. 2021), as well several other regions experiencing significant hellbender declines (Bodinof et al. 2012, Boerner 2014, Kenison and Williams 2018a,b). However, broad questions remain regarding the overall efficacy of head-starting and the best reintroduction strategies to rebuild viable Eastern Hellbender populations. Specifically, because many head-starting programs rely on the collection of wild egg masses, it is critical to assess trade-offs posed by head-starting from the standpoint of increasing initial survival vs. long-term population growth and persistence (Seddon 1999; Kissel et al. 2014).

The goal of this research was to evaluate the success of Eastern Hellbender head-starting efforts in Ohio and identify reintroduction strategies for rebuilding populations. Specifically, our objectives were to: (1) estimate survival of captive-reared Eastern Hellbenders released across three watersheds in eastern Ohio for up to three years post-release; (2) identify the best strategies for rebuilding populations using simulated population trajectories and survival rates derived from our field study and from existing literature, and (3) evaluate the efficacy (or value) of headstarting efforts by comparing survival to adulthood of captive-reared eggs relative to wild-raised eggs. For the first objective, we used passive integrated transponder (PIT)-tag surveys (Kraus 2015) of head-start Eastern Hellbenders conducted across three years (2018–2021) in eastern Ohio to parameterize Cormack-Jolly-Seber models (Cormack 1964; Jolly 1965; Seber 1965) and estimate annual and three-year apparent survival. For objective 2, we simulated different number of releases and frequency of release using demographic rates from existing literature (Unger et al. 2013) and from our data to estimate the number of released individuals reaching adulthood. For objective 3, we used survival rates of young wild and head-start animals and evaluated the number of eggs needed to reach a certain number of adults under wild and captive conditions. Cumulatively, these demographic data and population projections will inform future reintroduction actions aimed at recovering Eastern Hellbender populations in regions experiencing rapid declines.

**Methods**

Hellbender head-starting and releases – Hellbender head-starting efforts have been underway in Ohio since 2011, and this strategy has been the primary management method for population recovery in the state (Lipps et al. 2013). The Ohio Hellbender Partnership (OHP) leads this effort and consists of a collaborative team of stakeholders including biologists, zoo personnel, and land managers. Since the inception of this program, the OHP has released > 1,800 captive-reared Hellbenders into Ohio watersheds to augment or reestablish Ohio populations.
Hellbender husbandry – In 2018, a total of 205 animals were tagged using 12.5-mm 134.2 kHz RFID PIT tags (Table 1). Animals were raised at Columbus Zoo and Aquarium (CZA), Toledo Zoo (TZ), and Penta Career Center (PCC; Perrysburg OH) using husbandry methods standard for aquatic salamanders. Animals were hatched from eggs collected in the wild and raised in captivity until 3-4-years old. Hellbender egg clutches were collected from several Ohio nest sites in September of 2014 and 2015. The clutches were transported to CZA and TZ (and in time a subset of larvae were transferred to PCC), where long-term quarantine facilities dedicated to hellbender head-starting exist. Biosecurity best practices are followed within the head-start facilities to minimize the potential for amphibian pathogens to move between the institutions’ permanent animal collections and the captive hellbender populations (Pessier and Mendelson, 2017). Upon arrival at each zoo, the eggs were first separated by cutting the connecting strands between capsules with tissue scissors, then assessed for development and counted. Viable eggs were placed into aquaria specially modified to provide optimal water flow around the developing eggs. Most larvae hatched over a two-week span in early October each year.

Table 1
Summary of head-start Eastern Hellbender tagged with 12.5 mm PIT tags and released across 3 eastern Ohio streams. Surveys started after release in August 2018 and concluded in July 2021 for a total of three years post-release. Note: * sites surveyed between August 2018 and November 2019, and no animals recaptured after this date.

<table>
<thead>
<tr>
<th>Waterway</th>
<th>Site</th>
<th>Total</th>
<th>Years surveyed</th>
<th>Number of surveys</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Site A1</td>
<td>52</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Site A2</td>
<td>6</td>
<td>1.5*</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Site A3</td>
<td>33</td>
<td>1.5*</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>Site B1</td>
<td>26</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Site B2</td>
<td>35</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>Site C1</td>
<td>20</td>
<td>1.5*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Site C2</td>
<td>20</td>
<td>1.5*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Site C3</td>
<td>13</td>
<td>1.5*</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>205</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Newly hatched hellbender larvae were removed from the egg incubation aquaria and subsequently housed as groups in rearing tanks. Larvae were provided with artificial cover objects for refuge, such as lengths of PVC pipe and natural stone tiles. Larvae began ingesting food 1 ½ to 2 months after hatching, when yolk reserves had been absorbed. Diet items offered to head-start hellbenders included commercially-sourced live invertebrates and/or frozen crustacean and fish products. Diets were offered twice weekly in amounts approximating 2–4 percent of body weight. As larvae grew, they were distributed to additional rearing tanks to reduce animal density. Water quality was routinely monitored and water
changes were performed at weekly intervals using aged municipal water. Head-start hellbenders were periodically measured and weighed to evaluate body condition and growth. Animal welfare assessments were also routinely conducted. After the hellbenders reached 24 months of age, zoo staff tagged each animal by injecting a PIT tag intra-muscularly into the tail (Unger et al. 2012). In the weeks prior to release all animals were confirmed to be negative for chytrid amphibian fungus via PCR of skin swab samples.

Animal releases and PIT-tag surveys – Headstart animals were released across eight sites in three watersheds in eastern Ohio. The release sites were rock field reaches that ranged between 50 and 100 m in length, and the number of animals released per reach varied between 6 and 52 (Table 1). The release locations were selected based on previous habitat assessments and knowledge of Eastern Hellbender occupancy; release reaches were either previously unoccupied or have current occupancy by known wild adult individuals.

We conducted PIT tag surveys using an amplified PIT tag reader Biomark HPR Plus and a BP Plus Portable Antenna (Biomark Inc., Boise ID), which allowed us to detect 12.5 mm PIT tags from 31–42 cm through any medium (Fig. 1). We conducted PIT tag surveys by scanning the entire area of the release locations, as well as 20–30 m upstream and downstream. We did so by walking transversal transects and scanning all substrate and available rock cover 1-1.5 m on each side of the transect. When a tag was detected, the reader unit recorded the tag and logged the georeferenced location automatically. We spent additional time scanning all large cover rocks. We verified that the PIT tag detections were indeed detections of animals, not of loose tags. If a tag was encountered within substrate that was not a possible head-start shelter, we searched for the tag to confirm it; in such cases, we considered that either the tag was ejected, or mortality occurred. When water flow and depth allowed, we confirmed presence of animals via a borescope (Extech Instruments, Boston MA) or an Aqua-Vu Micro 5.0 Revolution Pro underwater camera (AquaVu, Crosslake, MN). We did not attempt to capture all animals as to minimize habitat disturbance, but we were able to capture several animals that were found sheltering under smaller, unstable rocks; we weighed, measured, and visually evaluated the body condition (e.g., bite marks, missing limbs) of the animals. Surveys started in August 2018 immediately post-release and continued through July 2021; during each year, we conducted surveys between August – November 2018, July – October 2019, July – November 2020, and June – July 2021. Animals were detected at all sites in 2018 but persisted at only three release sites throughout the study period (Table 1). For this analysis, we only used capture histories at these three sites (A1, B1, B2) across 25 surveys occasions (see details below).

**Capture-recapture analyses**

We built a capture recapture history spanning 25 survey occasions between August 2018 and July 2021. We used a Cormack-Jolly-Seber (CJS) open population model (Cormack 1964; Jolly 1965; Seber 1965) to estimate apparent survival of head-start hellbenders. CJS models use capture-recapture histories to develop maximum likelihood estimates of the probability of apparent survival ($\Phi$) and capture ($p$). The probability of apparent survival is the likelihood both that an individual survived, and that the individual did not permanently emigrate out of the monitoring area over the interval of time between sampling
periods. Our models account for unequal time periods between sampling sessions. Including the probability of capture in maximum likelihood models controls for the potential that an individual may survive yet evade capture during a sampling session. We implemented the CJS in MARK (White and Burnham 1999) via the program R, package ‘Rmark’ (Laake 2013).

We fitted a small set of candidate models to the data. Survival was modelled as either constant through time, varying through time or varying by site. The probability of recapture was modelled in the same way: constant, time-varying, or site-varying. We estimated survival probability using a model-averaging procedure across all models in our model set. Because surveys were conducted at intervals as narrow as one week (during the initial Fall 2018 post-release), we used ‘week’ as our sampling interval; as such, the survival estimates produced by our models were weekly survival. The releases occurred in August, therefore, we estimated yearly survival as the product of all survival estimates from one survey to the next starting with mid-August (i.e., August 2018 – July 2019, August 2019 – July 2020, and August 2020 – July 2021). As such, for our annual apparent survival estimates we used recapture histories that included 12, 5, and 8 surveys for each of the three periods. We also estimated the overall apparent survival during the study period as the product of all weekly survival probabilities adjusted for each individual inter-survey period. We estimated standard errors for the yearly and overall estimates of apparent survival using the delta method implemented in packages ‘msm’ (Jackson 2011) and ‘Rmark’ (Laake 2013).

**Population augmentation projections**

We simulated the trajectories of a newly established Eastern Hellbender population using an initial release of 3-year old head-started animals under a range of scenarios (Table 2). We started with a population of 100 head-start individuals, and subsequent releases occurred every 2 or 3 years using the same number of animals as the initial release. We used survival rates from our highest survival site to one-year post-release and for Year 1 to Year 2 and Year 2 to Year 3. For subsequent years we used survival rates extracted from literature (Unger et al. 2013) for subadults (Year 4 to Year 6; = animals up to 8 years old) and for adults (animals 9 + years of age). We conducted 1000 stochastic simulations for each scenario. For each simulation replicate, all survival rates were simulated using field and literature-based mean survival values (Table 4) with an additive term on a logit scale, drawn from a normal distribution of mean = 0 and standard deviation = 0.2 (denoting low-medium effects on survival; Popescu et al. 2012). We only simulated demographic stochasticity (i.e., stage-specific survival rates varied between replicates, but not within replicates). In the absence of sufficient data to estimate spatiotemporal process variance in transition (survival) rates, we assumed that the parameter ranges represented parameter uncertainty, and were not a result of annual variation in environmental conditions (i.e., no environmental stochasticity). This approach is conservative in that it represents a worst-case scenario that maximizes the resulting estimated risk of extinction or decline.
Table 2
Parameters used to simulate reintroduced Eastern Hellbender population trajectories under a variety of scenarios.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of initial population and subsequent releases</td>
<td>100 individuals</td>
<td>Number of 3-year old animals released</td>
</tr>
<tr>
<td>Release frequency</td>
<td>2 or 3 years</td>
<td>Population augmentation occurs every 2 or 3 years</td>
</tr>
<tr>
<td>Number of releases (including initial release)</td>
<td>2 or 3</td>
<td>Number of reintroduction efforts (using 3-year-old head-start animals)</td>
</tr>
<tr>
<td>Subadult survival post-release (to year 1); animals are 4 years old</td>
<td>$0.40 \pm N(0,0.2)$</td>
<td>Mean survival $\pm$ additive term on a logit scale, drawn from a normal distribution of mean = 0 and standard deviation = 0.2 (based on our surveys)</td>
</tr>
<tr>
<td>Subadult survival post-release (year 1 to year 2); animals are 5 years old</td>
<td>$0.70 \pm N(0,0.2)$</td>
<td>Mean survival $\pm$ additive term on a logit scale, drawn from a normal distribution of mean = 0 and standard deviation = 0.2 (based on our surveys)</td>
</tr>
<tr>
<td>Subadult survival post-release (year 2 to year 5); animals 6–8 years old</td>
<td>$0.70 \pm N(0,0.2)$</td>
<td>Mean survival $\pm$ additive term on a logit scale, drawn from a normal distribution of mean = 0 and standard deviation = 0.2 (based on our surveys)</td>
</tr>
<tr>
<td>Adult survival (year 6 onwards); animals 9 years and older</td>
<td>$0.95 \pm N(0,0.2)$</td>
<td>Mean survival $\pm$ additive term on a logit scale, drawn from a normal distribution of mean = 0 and standard deviation = 0.2 (based on (Unger et al. 2013))</td>
</tr>
</tbody>
</table>

We ran the simulations for a 15-year period (14 transitions) and calculated the total Eastern Hellbender population for each replicate, as well as the number of individuals surviving to adulthood (9 + years of age, assuming that all animals were introduced when 3-years old in average). We did not consider reproduction and population dynamics post-reproduction for two reasons: (1) population projections for hellbenders have been implemented by (Unger et al. 2013), and (2) it was outside the scope of this analysis, as we assume that successful reproduction occurs once animals reach maturity [otherwise reintroduction efforts cannot lead to sustainable populations]. All simulation routines and statistical analyses were performed using R v 4.0.3 (R Core Team 2021).

Using wild eggs for captive-rearing and reintroductions

We compared the number of Eastern Hellbender eggs required to establish a population naturally versus via head-starting. Specifically, we asked what is the value of hatching an egg mass in captivity under controlled conditions compared to allowing egg masses to naturally develop in the wild?

We assumed that once wild eggs are collected for captive-rearing and raised to 3-years old, they have 100% survival in captivity, but similar calculations can be done by varying survival in captivity. We used the stage-based survival rates from wild animals (Unger et al. 2013) and our head-start monitoring to back-calculate the number of eggs required to reach the number of adult individuals surviving estimated based on a single release of 100 animals. Thus, we simulated 3 deterministic (no demographic
stochasticity) scenarios: (1) survival rates in wild populations (Unger et al. 2013), (2) mean stage-based survival rates from our surveys across all three sites, and (3) site-specific estimates of survival estimates. We calculated the mean adult population estimate at time 10 (individuals reaching maturity at age 9) for each of the four head-start release scenarios (mean across all three sites and each site separately), and calculated the number of eggs required to reach those abundances from wild eggs (in the absence of head-starting) using the stage-based survival rates from (Unger et al. 2013). Lastly, we calculated the ratio of wild-developing eggs to the number of eggs required for head-starting to evaluate the biological efficacy of the head-start program for rebuilding hellbender populations.

**Results**

The animals released in 2018 (Table 1) were 29.10 ± 0.55 cm long (with a mean Snout-Vent Length = 16.67 ± 0.42 cm) and weighed 119.80 ± 6.33 g. Sixteen animals were recaptured and measured in 2019 and 2020. These animals (N = 16) were 31.72 ± 0.36 cm in total length (19.93 ± 0.36 cm SVL), weighed 188.12 ± 8.61 g, and overall had good body condition (i.e., no wounds, lacerations, or missing limbs).

**Post-release apparent survival of head-start hellbenders**

The best CJS model was a complex model with survival as a function of time, and recapture as a function of site and time (Table 3), and we estimated apparent survival and recapture probability using a model averaging procedure across all models. The three sites had similar apparent survival across the 3-year study period (Table 4), with the highest at site B1 (0.166 ± 0.147), and lowest at site B2 (0.149 ± 0.145). The overall survival across all sites estimated via model averaging was 0.162 ± 0.0.61 (Table 2). Apparent survival was the lowest in the first year post-release (0.383 ± 0.058 across all sites) and increased during years two and three post-release (0.696 ± 0.086 and 0.609 ± 0.154, respectively).
Table 3
CJS models used to evaluate apparent survival of head-start Eastern Hellbenders released in 3 Ohio streams. \( \Phi \) = apparent survival; \( p \) = recapture probability; \( K \) = number of parameters, AICc = Akaike Information Criterion adjusted for small sample size.

<table>
<thead>
<tr>
<th>Model</th>
<th>K</th>
<th>AICc</th>
<th>( \Delta \text{AICc} )</th>
<th>AICc weight</th>
<th>Deviance</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Phi(\sim \text{time}) ) ( \text{p}(\sim \text{site + time}) )</td>
<td>52</td>
<td>1773.064</td>
<td>0.000</td>
<td>0.611</td>
<td>936.264</td>
</tr>
<tr>
<td>( \Phi(\sim 1) ) ( \text{p}(\sim \text{site + time}) )</td>
<td>28</td>
<td>1775.299</td>
<td>2.235</td>
<td>0.199</td>
<td>993.039</td>
</tr>
<tr>
<td>( \Phi(\sim \text{site + time}) ) ( \text{p}(\sim \text{site + time}) )</td>
<td>54</td>
<td>1776.554</td>
<td>3.490</td>
<td>0.106</td>
<td>935.013</td>
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<tr>
<td>( \Phi(\sim \text{site}) ) ( \text{p}(\sim \text{site + time}) )</td>
<td>30</td>
<td>1777.832</td>
<td>4.768</td>
<td>0.056</td>
<td>991.186</td>
</tr>
<tr>
<td>( \Phi(\sim \text{time}) ) ( \text{p}(\sim \text{time}) )</td>
<td>50</td>
<td>1779.937</td>
<td>6.873</td>
<td>0.019</td>
<td>947.847</td>
</tr>
<tr>
<td>( \Phi(\sim \text{site + time}) ) ( \text{p}(\sim \text{time}) )</td>
<td>52</td>
<td>1782.884</td>
<td>9.820</td>
<td>0.004</td>
<td>946.084</td>
</tr>
<tr>
<td>( \Phi(\sim \text{time}) ) ( \text{p}(\sim \text{site}) )</td>
<td>28</td>
<td>1786.488</td>
<td>13.424</td>
<td>0.000</td>
<td>1004.228</td>
</tr>
<tr>
<td>( \Phi(\sim 1) ) ( \text{p}(\sim \text{time}) )</td>
<td>26</td>
<td>1786.599</td>
<td>13.535</td>
<td>0.000</td>
<td>1008.696</td>
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<tr>
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<td>28</td>
<td>1788.427</td>
<td>15.363</td>
<td>0.000</td>
<td>1006.167</td>
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<tr>
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<td>30</td>
<td>1790.064</td>
<td>17.000</td>
<td>0.000</td>
<td>1003.419</td>
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<tr>
<td>( \Phi(\sim \text{time}) ) ( \text{p}(\sim 1) )</td>
<td>26</td>
<td>1792.834</td>
<td>19.770</td>
<td>0.000</td>
<td>1014.931</td>
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<tr>
<td>( \Phi(\sim \text{site} + \text{time}) ) ( \text{p}(\sim 1) )</td>
<td>28</td>
<td>1799.338</td>
<td>26.274</td>
<td>0.000</td>
<td>1017.077</td>
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<tr>
<td>( \Phi(\sim 1) ) ( \text{p}(\sim \text{site}) )</td>
<td>4</td>
<td>1812.269</td>
<td>39.205</td>
<td>0.000</td>
<td>1080.533</td>
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<tr>
<td>( \Phi(\sim \text{site}) ) ( \text{p}(\sim \text{site}) )</td>
<td>6</td>
<td>1814.352</td>
<td>41.288</td>
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<td>1078.548</td>
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<td>( \Phi(\sim 1) ) ( \text{p}(\sim 1) )</td>
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<td>1820.185</td>
<td>47.121</td>
<td>0.000</td>
<td>1092.493</td>
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<tr>
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<td>1821.680</td>
<td>48.616</td>
<td>0.000</td>
<td>1089.944</td>
</tr>
</tbody>
</table>

Recapture probability varied across the survey occasions from < 0.10 to ~ 1.00 (Fig. 2); recapture probabilities were lowest at site B2 across the study (by 0.15–0.20 for each recapture event; this site was deeper, with large areas of rock piles and more opportunities for evading detection).
Table 4
Mean apparent survival estimates (± 1 standard error) of head-start Eastern Hellbenders released in 3 Ohio stream reaches, 2018–2021.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A1</td>
<td>0.405 ± 0.113</td>
<td>0.660 ± 0.136</td>
<td>0.575 ± 0.308</td>
<td>0.154 ± 0.144</td>
</tr>
<tr>
<td>Site B1</td>
<td>0.420 ± 0.118</td>
<td>0.672 ± 0.134</td>
<td>0.588 ± 0.289</td>
<td>0.166 ± 0.147</td>
</tr>
<tr>
<td>Site B2</td>
<td>0.399 ± 0.116</td>
<td>0.655 ± 0.139</td>
<td>0.571 ± 0.322</td>
<td>0.149 ± 0.145</td>
</tr>
<tr>
<td>All sites</td>
<td>0.383 ± 0.058</td>
<td>0.696 ± 0.086</td>
<td>0.609 ± 0.154</td>
<td>0.162 ± 0.061</td>
</tr>
</tbody>
</table>

Two sites – B1 and B2 – were separated by ~ 3 km, and we did not observe movements between the two sites. In addition, the B1 release site had a large, deep pool in the middle section (~ 20 m reach); animals released on each side of this pool were found to stay at the release sites throughout the study period, except for one individual released upstream from the pool and was recaptured downstream from it.

Population augmentation projections

Using survival probabilities estimated from this study and from Unger et al. (2013), we found that the number of adults at 15 years after the first reintroduction was greater under the three releases scenario compared to the two releases scenario, and that releasing animals every three years vs every two years has a small effect on the number of surviving adults (Fig. 3). In general, the maximum number of adults 15 years post-release of batches of 100 3-years old individuals was 21 for two releases and 36 for three releases, but they could be as low as 6 and 11 individuals, respectively. There was only a small difference between releases every two and three years, with a slightly larger number of adult animals available 15 years post-release under the three-year scenario (Fig. 3).

Head-starting vs. natural reproduction

We calculated the tradeoffs between head-starting vs wild reproduction by comparing the number of eggs that would be needed to reach the same number of reproductively active adults under captive-rearing and wild conditions (Table 5). Overall, if 100 eggs were collected from the wild, had 100% survival during head-starting, and had mean annual survival to adulthood post-release based on our study and estimates from Unger et al (2013), the number of 9-year-old animals reaching adulthood would be 8.5. To reach the same number of 9-years-old adults from eggs hatched in the wild using survival rates from Unger et al. (2013), we would need 710 eggs (Table 5). In other words, one egg mass in hand is worth 7.1 in the stream. If we calculate these ratios separately for each of the 3 reintroduction sites, the ratios of wild:captive eggs are: 6.5 (for Site A1), 7.0 (for Site B1) and 6.3 (for Site B2) (Table 5).
Table 5
Comparison of survival rates of head-started individuals and wild counterparts and their impact on survival to adulthood. The ratios of wild to head-started eggs illustrate that 6–7 times more eggs that hatch and develop in the wild are needed to reach the same number of adults, compared to a scenario where eggs were collected and raised in captivity. For example, assuming no mortality during head-starting, 710 eggs in the wild = 100 eggs raised in captivity (under the General head-start population).

<table>
<thead>
<tr>
<th>STAGE / AGE</th>
<th>Wild populations (Unger et al. 2013)</th>
<th>General head-start population</th>
<th>Site A1 head-start population</th>
<th>Site B1 head-start population</th>
<th>Site B2 head-start population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg/larvae to 1-yr old</td>
<td>0.100</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>1–2</td>
<td>0.750</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>2–3</td>
<td>0.750</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>3–4</td>
<td>0.750</td>
<td>0.400</td>
<td>0.405</td>
<td>0.420</td>
<td>0.399</td>
</tr>
<tr>
<td>4–5</td>
<td>0.750</td>
<td>0.700</td>
<td>0.660</td>
<td>0.672</td>
<td>0.655</td>
</tr>
<tr>
<td>5–6</td>
<td>0.750</td>
<td>0.600</td>
<td>0.575</td>
<td>0.588</td>
<td>0.571</td>
</tr>
<tr>
<td>6–7</td>
<td>0.750</td>
<td>0.750</td>
<td>0.750</td>
<td>0.750</td>
<td>0.750</td>
</tr>
<tr>
<td>7–8</td>
<td>0.750</td>
<td>0.750</td>
<td>0.750</td>
<td>0.750</td>
<td>0.750</td>
</tr>
<tr>
<td>8–9 (adulthood)</td>
<td>0.950</td>
<td>0.950</td>
<td>0.950</td>
<td>0.950</td>
<td>0.950</td>
</tr>
</tbody>
</table>

Wild eggs : Eggs raised in captivity
7.1 6.5 7.0 6.3

Discussion
We evaluated apparent survival of headstart hellbenders released in three Ohio streams for three years. While overall survival was relatively low across the study period (Table 4), the annual survival matched our expectations, with lower survival during the first-year post-release (2019) and higher survival in subsequent years (2020 and 2021). Animals recaptured by hand in subsequent years showed considerable growth and overall excellent body condition. Our population projections using survival data from this study and existing literature suggest that building hellbender populations requires successive reintroductions; three reintroductions every three years (at 100 per cohort per site) produced the fastest and highest number of individuals attaining maturity (Fig. 3). We recommend repeated releases on fewer sites than releasing smaller cohorts at many sites only once or twice when the number of available animals is limited. Overall, for our study system and other localities dealing with similar recruitment issues, headstarting can be an effective tool for rebuilding hellbender populations, provided that stream...
and habitat quality at release sites are amenable for natural reproduction and development. Specifically, collecting and captive rearing wild eggs resulted in up to seven times more animals reaching adulthood after headstarting efforts compared to natural reproduction and egg development (Table 5).

Survival was lowest in the first year post-release. This was expected given that animals were naïve relative to the natural environment, and corroborates findings from other amphibian release studies (Stamps and Swaisgood 2007, Germano and Bishop 2009). Some hellbender reintroductions have been ‘soft releases’ (Boerner 2014, Kraus et al. 2017, McCallen et al. 2018, Kocher 2019). For example, Eastern Hellbender releases in the Allegheny River drainage in New York (Boerner 2014, Kocher 2019) have included soft releases using both artificial nest boxes (Briggler and Ackerson 2012) and wire mesh cages. Kocher (2019) suggested that soft release techniques had no effect on post-release movement, but may improve short-term post-release survival via refuge from predators. Additionally, recent work has suggested that environmental conditioning may improve both swim performance (Kenison and Williams 2018a) and predator-recognition (Crane and Mathis 2011; Kenison and Williams 2018b) in captive-reared hellbenders.

Our results on relatively low survival over three years are conservative. The capture-recapture models used here do not differentiate between mortality and emigration; thus, headstart animals could have left the release sites and avoided subsequent detection. This is a common pitfall for amphibian translocations, and one of the main causes for failed introduction or reintroduction efforts (Germano and Bishop 2009). Wild hellbenders are sedentary, typically spending most of their time within 30–40 m of the same stream reach (Nickerson and Mays 1973, Foster et al. 2009). Headstart hellbenders fitted with telemetry units and tracked for short time periods have been shown to have lower site fidelity (60%) than their adult resident (100%) and translocate (90%) counterparts (McCallen et al. 2018). Bodinof et al. (2012) showed that Missouri headstarts dispersed within 550 m of release sites with most individuals dispersing < 50 m. Boerner (2014) and Kocher (2019) estimated average (± SE) cumulative movement distances for New York headstarts at 653 ± 138 m and 1102 ± 267 m, respectively, and Kraus (2017) documented a captive reared individual moving ~ 1.3 km from its initial release site. Additionally, headstarts typically move downstream (Bodinof et al. 2012b, Boerner 2014, Kraus et al. 2017, McCallen et al. 2018, Kocher 2019), likely due to their naïvety to high flow events. In Ohio, hellbenders have disappeared from > 80% of their range since the 1980s, and populations are sparse, spatially segregated, and have low abundance (Lipps 2011; Smeenk et al. 2021). As such, if animals leaving the release sites do not return, they are unlikely to contribute to the reproducing population are effectively ‘dead’ from a demographic perspective. Thus, we propose that releasing animals at multiple reaches within a stream, and, when possible, ensuring that release sites are adjacent to other suitable downstream habitat (Boerner 2014, Kocher 2019) is a better strategy than releasing animals at isolated reaches.

From a temporal perspective, our population simulations show that rebuilding hellbender populations is a long-term process, and that adequate post-release monitoring of animals should be undertaken to evaluate the success of reintroduction efforts. Given the low survival post-release, we found that releasing multiple cohorts every 2–3 years represents a good alternative to single releases. Repeated
releases (every 3 years) of a relatively large number of individuals (N = 100), yielded 11–36 reproductive adults 15 years after the first introduction. Thus, our findings meet recommendations of Germano and Bishop (2009) for large cohorts to be released at any given location. The population models used here, as well as the underlying assumption of establishing populations, assume that reintroduced animals will reproduce once they reach a suitable age structure (4–8 years; Peterson et al. 1983) and that the threats that affect reproductive success and survival of sensitive life stages are removed.

The goal of headstarting is to improve survival of animals during critical (or at risk) life stages, and there is much uncertainty about the demographic outcomes of releasing one stage or another. For example, (Germano and Bishop 2009) found that the success for translocations varied across amphibian species based on whether the life stages were eggs, larvae, or juveniles. However, the most important predictor for translocation success was the number of animals released, with relocations of > 1000 animals being most successful. One of the few studies that evaluated the demographic implications of releasing different life stages for endangered amphibians (Kissel et al. 2014) concluded that translocations involving Oregon spotted frogs (Rana pretiosa) yielded higher population growth and lower risk of extinction when captive-breeding and release of larvae were implemented as opposed to headstarting animals and releasing them as juveniles. In the case of hellbenders, captive breeding success has been low; Ozark hellbenders (C. alleganiensis bishopi) have been bred in captivity for the first time at St. Louis Zoo (Missouri) in 2011 (Ettling et al. 2013), and more recently at the Mesker Park Zoo (Indiana). In addition, there is limited information on survival of larvae post-hatching, or the pathways through which different perceived threats (e.g., low riparian forest cover (Bodinof Jachowski and Hopkins 2018), high conductivity (Pitt et al. 2017), siltation (Unger et al. 2021)) affect different life stages. Thus, efforts to captive-raise animals as long as possible and release large cohorts are likely to continue to be the most plausible recovery strategy for this species, while mechanisms of suppressed recruitment are further investigated.

Our analysis of the efficacy of captive-raising animals from wild nests for headstarting vs. allowing natural development of eggs highlights the important role of raising animals in captivity for 3–4 years. We found that to reach the same population size of adult hellbenders, captive rearing is up to 7 times more efficient than allowing eggs to develop naturally. Thus, considering the assumption of no or little mortality during captive rearing, this strategy may be useful to increase recruitment in declining populations experiencing reduced reproductive success. The analogy of “1 egg in hand is worth 7 in the stream” can be seen as optimistic, but hellbender husbandry is relatively well understood as many zoos and aquaria across North America have state-of-the-art facilities and resources devoted to amphibian reintroduction programs, including hellbender headstarting. This particular analysis is subject to assumptions related to natural survival rates of various age classes, some of which can only be inferred from prior studies (Unger et al. 2013). For example, larval survival in natural settings and survival of young animals (between larval stage and first reproduction) are notoriously difficult to assess for cryptic species (Foster et al. 2009, Diaz et al. 2022). While some progress has been made in understanding the demography of early stages via monitoring wild hellbender nests in artificial nest boxes (Briggler and Ackerson 2012, Bodinof Jachowski et al. 2020, Button et al. 2020, M. Kaunert, unpubl. data), we still lack
basic natural history knowledge of this species or the threats to its persistence and their potential for mitigation.

The Eastern Hellbender is a species in need of active management and conservation throughout much of its range, even though the majority of the declining populations are not being warranted protection through the Endangered Species Act. Thus, improving the success of headstarting programs is essential for the persistence of the species across its geographic range. Our results suggest that despite relatively low survival of headstart animals following release, headstarting can be an effective recovery strategy provided it achieves the ultimate goal of re-establishing self-sustaining populations. PIT-tag monitoring offers a minimally invasive, time- and cost-effective method for long-term monitoring of large cohorts of released hellbenders (Kraus 2015), and could represent a valuable component for measuring success of future recovery efforts in regions where headstart efforts are implemented. Future work should investigate the effect of emerging conservation strategies (artificial nest boxes, soft release techniques, pre-release conditioning) on the survival and persistence of reintroduced cohorts. Re-establishing populations of a cryptic, long-lived organism such as the Eastern Hellbender presents many challenges, as the success of various stream or catchment-level management actions to reverse population declines has not been fully assessed. Therefore, improving practices for headstarting will be vital to properly allocate management resources while threats to hellbender populations are further understood and mitigated.

Declarations

Acknowledgments

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Ethical approval: This work was conducted under Ohio University Institutional Animal Use and Care Committee (IACUC) protocol 18-L-007.

Competing interests: No competing interests

Author contributions: MK and VP wrote the bulk of the manuscript; PJ contributed headstart animals and husbandry methods; MK and VP analyzed the data; MK, VP and RB conducted fieldwork and collected the data; SS and VP developed the project idea and initiated the research; all authors contributed edits and reviewed the manuscript.
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**Availability of data and materials:** Raw data for capture-recapture analysis can be made available upon request. The R code for running population simulations is available as Supplementary Material associated with this manuscript.

**References**

28. Kenison EK (2018) ADVANCING EASTERN HELLBENDER CONSERVATION THROUGH NOVEL HEAD-STARTING TECHNIQUES by Department of Forestry and Natural Resources


1669.1


60. Unger SD, Goforth RR, Rhodes OE Jr, Floyd T (2021) Short-term exposure to elevated suspended sediment increases oxygen uptake of gilled larval Eastern Hellbender (Cryptobranchus alleganiensis)

Figures

Figure 1

Figure 2

Variation of recapture probability across the study period at each survey location predicted by CJS models; dots: Site A1, triangles: Site B1, squares: Site B2
Figure 3

Simulated total and adult Eastern Hellbender population trajectory under various head-start release scenarios: an initial release of 100 3-year-old individuals followed by 1 or 2 subsequent releases of 100 individuals every 2 or 3 years. The number of adults at Year 15 is presented in parentheses for each scenario: a) 2 releases 2 years apart (mean = 12 adults, range = 6 – 19), b) 2 releases 3 years apart (mean = 13 adults, range = 8 – 21), c) 3 releases 2 years apart (mean = 20 adults, range = 11 – 31), and d) 3 releases 3 years apart (mean = 20 adults, range = 11 – 36). Demographic stochasticity was simulated using parameters in Table 2.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Rcodeforstochasticpopulationsimulations.docx