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Juvenile Eastern Hellbender (*Cryptobranchus a. alleganiensis*) Preference Among Juvenile Hut Designs

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***Abstract*.—Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) are fully aquatic, giant salamanders occurring throughout the eastern U.S. In the Blue River, in southern Indiana, Hellbender habitat is sporadic with extensive patches of low-quality habitat separating high-quality sites. Artificial Hellbender nest boxes placed in rivers are successful for egg collection and shelter for adults but are not suitable for juveniles. Rearing Hellbenders in captivity is both time and resource intensive and maximizing post-release survival is important. We constructed juvenile shelters (huts) with different cavity volumes and entrance heights to test for preferences in captivity with the intent to provide shelter for juveniles following reintroduction. Our study included two phases. In the first phase, we tested preference for different cavity volumes. In the second phase, we tested preference for different entrance heights. We hypothesized that juvenile Hellbenders would show a preference for shelter type. We predicted that they would prefer smaller cavity volumes and smaller entrance heights. Significantly more Hellbenders occurred in huts with smaller cavity volume throughout phase 1. More Hellbenders occurred in huts with smaller entrances throughout phase 2, but the number of animals in small-entrance huts and the proportion of occupied, small-entrance huts declined over time compared to large entrances. Deploying these juvenile huts may be a critical action for Hellbender reintroductions to increase available shelter at stopover sites and decrease exposure to predators as juveniles disperse between high-quality habitats.**

Key Words.—amphibian declines; aquatic salamander; Caudata; conservation; endangered; post-release survival; salamanders; shelter

Introduction

Amphibian species are important indicators of environmental health (Collins and Storfer 2003) but are rapidly declining worldwide with up to 41% of identified species currently facing extinction (Houlahan et al. 2000; Stuart et al. 2004; International Union for Conservation of Nature [IUCN] 2021). Anthropogenic disturbances such as climate change, habitat degradation, disease, and agricultural runoff (i.e., sedimentation, nutrients, pesticides) negatively affect amphibian populations on a large scale (Blaustein et al. 1994), including the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*; hereafter, Hellbenders). Hellbenders are long-lived, fully aquatic salamanders spending their entire lives in rivers and streams. This entirely aquatic lifestyle combined with their highly permeable skin, cutaneous respiration, and reliance on coarse, unembedded substrates make them an important indicator species in the stream ecosystem (Pugh et al. 2016; Bodinof Jachowski and Hopkins 2018; Unger et al. 2020).

Hellbenders are habitat specialists that require well-oxygenated, high-flow rivers and streams with loose gravel, cobble, or bedrock substrate with large boulders for shelter (Williams et al. 1981; Olson et al. 2012). Sediment runoff increases turbidity and temperature of lotic habitats while decreasing interstitial spaces between coarse substrates and filling cavities under shelter rocks. Increased sedimentation decreases the ability of Hellbenders to find cover (Fobes 1995; U.S. Fish and Wildlife Service 2018) and is thought to be a major contributing factor to Hellbender population declines, especially in agricultural areas of the Midwest and the southern U.S. (Erin Crone, unpubl. report).

In Indiana, Hellbenders are state-listed as Endangered and have been extirpated from all historic rivers except the Blue River in southern Indiana, USA (Kern 1984; Burgmeier et al. 2011b); however, even within the Blue River, the population has experienced a considerable decline over the last decade (Burgmeier et al. 2011b; Unger et al. 2013). The ultimate cause of decline in the Blue River is unknown, but it is thought to have been due to a decline in water quality and increase in sedimentation resulting from high levels of agriculture and limited conservation practices in the mid to late 20th Century. This has led to a low density population consisting of only older age class individuals with very few opportunities for reproduction (Burgmeier 2011b). Current water quality and habitat availability data suggest that these issues have been reversed and the river is suitable for Hellbender habitation and reintroduction (Burgmeier 2011a).

To reverse declining trends and bolster the population, Purdue University, West Lafayette, Indiana, and the Indiana Department of Natural Resources have conducted Hellbender head-starting and reintroduction programs to increase population sizes (Kenison et al. 2016; Kraus et al. 2017; Kenison and Williams 2018; McCallen et al. 2018). Following head-starting, which includes collecting and rearing wild caught eggs in captivity, release sites are then selected and 4–5-y-old, captive-reared juveniles are released into high-quality habitat deemed suitable for the species (McCallen et al. 2018). Survivorship through 1 y for captive-reared juveniles released into the Blue River was previously reported as 52.6% (Kraus et al. 2017), but recent releases in the Blue River report survival ranging between < 44% to as high as 74% through one year depending on the season of release and whether individuals were raised in high-flow versus no-flow conditions in captivity (Nicholas Burgmeier et al. unpubl. data).

Suitable release habitat for Hellbenders in the Blue River is not continuous. Suitable release habitat is connected by silty, low-quality habitat such that individuals moving between higher-quality habitats face increased predation risk due to fewer interstitial spaces and cover rocks. The deployment of artificial shelters has become a common practice for conservation of adult Hellbenders (Briggler and Ackerson 2012; Bodinof Jachowski et al. 2020). They have been effective at providing supplemental shelter (up to 48% shelter occupancy) in areas with high densities of Hellbenders but low habitat density (Bodinof Jachowski et al. 2020) and at providing habitat for nesting (Button et al. 2020a). There are few studies focused on juvenile Hellbender artificial shelter preferences, however, despite reintroductions of juveniles occurring in several states such as West Virginia and New York in the U.S. (Jensen 2013; Greathouse 2015).

Captive-reared juveniles released in the Blue River have a lower probability of using the standard, adult-sized artificial nest rocks, and disperse downstream more frequently than wild adults reported in other studies (Bodinof Jachowski 2016; McCallen et al. 2018). In North Carolina, USA, adult Hellbenders selected natural shelters that had flat bottoms, larger cover rocks, and deeper cavities that could provide more protection from predators (Rossell et al. 2013). Most of the natural shelters in North Carolina also had a single entrance oriented downstream (Rossell et al. 2013). In Tennessee, USA, the size of selected shelters differed among Hellbender age classes. The mean lengths for adult and juvenile shelters were 794 ± 254 (standard deviation) mm and 686 ± 252 mm, respectively, suggesting an ontogenetic shift in microhabitat selection (Hecht et al. 2019). Although information about adult habitat use is frequently reported, preferences of juveniles are less often reported. Rossell et al (2013) reported there were no differences between shelter rock size or cavity depth used by wild adults and juvenile Hellbenders; however, Freake and Deperno (2017) and Da Silva Neto et al. (2019) both reported wild subadult Hellbenders using smaller cover objects than adults. Similarly, McCallen et al. (2018) reported that post-release, captive-reared juveniles used smaller shelters than wild adults. We also have observed only a single post-release, radio-tagged, captive-reared juvenile (of 119 released) briefly occupying an adult-sized artificial nest rock over the course of approximately one year of radio-tracking, despite numerous available unused nest boxes present near the release location and other occupied rocks (unpubl. data). Therefore, it would be useful to determine shelter dimension preferences of captive-reared juveniles, and possibly increase reintroduction success, by installing shelters for juveniles *in situ* to increase survival following reintroductions.

We constructed juvenile huts (hereafter referred to as huts) with various characteristics for juvenile Hellbenders and tested their preference for huts with different cavity volumes (i.e., the volume of available space within the hut) and entrance heights (i.e., the height of the opening between the substrate and the top of the entrance). Understanding the preference of juvenile Hellbender for either of these fundamental shelter characteristics and slight variations might affect how they are used. Cavity volumes that are too large or too small might deter Hellbenders by the juveniles being too exposed or too confined, respectively. Similarly, differences in entrance height might be perceived by juveniles as offering more or less protection from potential predators. We hypothesize that juvenile Hellbenders will have a preference in hut construction. We predict that juvenile Hellbenders will select huts with smaller cavity volume and smaller entrance height. We sought to identify preferred characteristics for huts ex situ to prepare for future research focusing on increasing post-release juvenile survival by deploying huts into less desirable habitat within the Blue River. Artificial juvenile huts could potentially increase post-release survival of captive-reared juvenile Hellbenders, and overall success of recovery efforts, by providing shelter for individuals dispersing through low-quality habitats.

Materials and Methods

Study area and animals.—We conducted our study at the Aquaculture Research Lab (ARL) of Purdue University in West Lafayette, Indiana, USA. We constructed a 9.14-m long and 1.22-m wide flow-through artificial raceway with gravel substrate and flowing 14° C water designed to resemble natural Hellbender habitat. This raceway is larger, but similar in design, to that described in Kenison and Williams (2018). The raceway was in a temperature-controlled room and lights were on a 14:10 h light:dark cycle. We included 47 juvenile Hellbenders in phase 1 and 46 in phase 2 of the project. We used the same individuals in each phase; however, we removed one Hellbender between phases due to injury. We collected all Hellbenders in our experiment as eggs from the Blue River in 2015 and captive reared them at ARL. The average weight (g) and total length (mm) of study animals at the beginning of the study was 117 ± (standard deviation) 42 g (range, 43–275) and 296 ± 28 mm (range, 230–380), respectively.

Juvenile hut design.—Each phase of our experiment lasted 10 weeks, for a total of 20 weeks of experiment, with a two-week acclimation period between the two phases (phase 1: 20 April to 25 July 2020; phase 2: 10 August to 16 October 2020). In phase 1, we tested preferred hut cavity volume of juvenile Hellbenders comparing between small and large volumes (9,315.9 cm^3 and 15,526.5 cm^3, respectively). We constructed 12 huts for this phase, six small huts that were 7.62 cm tall, and six large huts that were 12.7 cm tall (Figs. 1 and 2). Entrance height of all huts was 3.81 cm for phase 1. In phase 2, we modified the entrance height, but used the preferred cavity volume from phase 1. Six huts had an entrance height of 3.81 cm and the other six had an entrance height of 6.35 cm. The entrance width, the width from one wall of the entrance to the opposite wall, for both phases was 10.16 cm.

To create the mold for the huts, we used a Sterilite Clearview Latch tub (43.2 × 28.3 × 16.5 cm; Sterilite Corporation, Townsend, Massachusetts, USA) and centered it within a larger Sterilite Clearview Latch tub (60 × 41 × 33.7 cm), creating a cavity space. Next, we applied non-stick mold release spray within the larger tub and on the outside of the smaller tub. We positioned a cinder block within the smaller tub to prevent the concrete from leaking underneath. We mixed crack resistant concrete mix (fiber reinforced) in a 19 L bucket and poured it between the two latch tubs until the desired height was reached (12.7 cm or 7.62 cm). Next, we wrapped a wooden block (10.16 × 10.16 × 3.81 cm or 6.35 cm, depending on desired entrance height) in a plastic bag, sprayed it with silicone release spray, and placed it within the mold to create the entrance.

To make the lid, we mixed concrete using the same procedure as above and poured a 2.54-cm thick layer on the bottom of the larger tub. While the concrete was wet, we placed two brushed nickel cabinet pull handles inside the mold for ease of handling when deployed in the raceway. We placed lids on top of huts to prevent any light from entering. Lastly, after curing, they were placed and soaked in water for one week to remove concrete residue prior to placement in the raceway.

Artificial raceway.—We placed huts on a 2.5–5.0 cm diameter gravel substrate in two alternating rows of six for each phase (Fig. 3). In phase 1, we tested

Figure 1. Top view of Eastern Hellbender (*Cryptobranchus a. alleganiensis*) juvenile huts. On the left is a hut with the lid off (with juveniles inside) and on the right is with the lid on.

Figure 2. Anterior view of Eastern Hellbender (*Cryptobranchus a. alleganiensis*) juvenile huts looking upstream in the raceway. Note entrances to huts on end view.

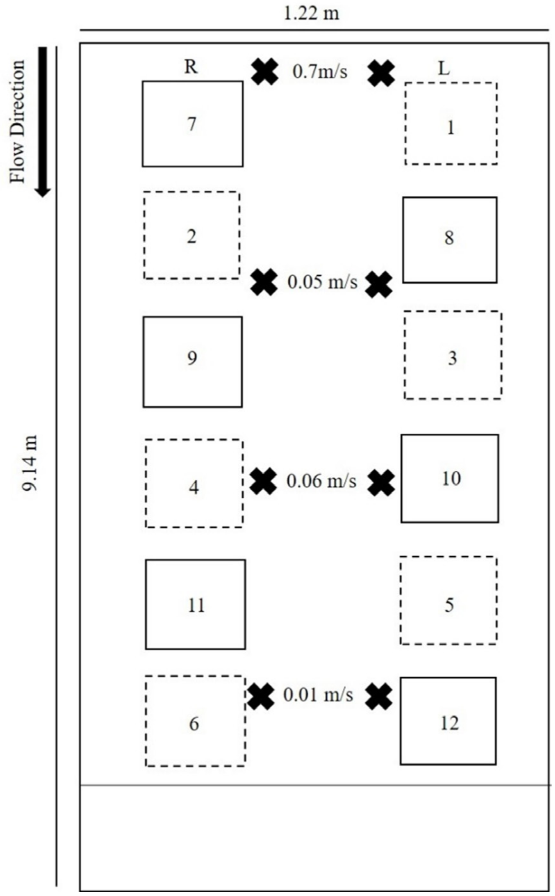

Figure 3. Hut placement for Juvenile Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) inside a large raceway at the Aquaculture Research Lab of Purdue University located in West Lafayette, Indiana, USA. Solid borders represent larger-cavity-volume juvenile huts (15,526.5 cm^3) with a height of 12.70 cm and an entrance height of 3.81 for phase 1, and for phase 2, a cavity volume of 9,315.9 cm^3 with a height of 7.62 cm and an entrance height of 5.08 cm. Dashed borders represent smaller-cavity-volume juvenile huts (9,315.9 cm^3) with a height of 7.62 cm and entrance height of 3.81 for phase 1 and 2. Numbers (1-12) located within juvenile huts represent the designated number for that individual juvenile hut. The letters R and L indicate which juvenile huts are on the right and left when looking downstream and bolded Xs indicate the water inflow locations into the artificial raceway. The numbers between the inflow locations are the water velocity (m/s).

between large and small hut cavity volumes. The first row was set up as alternating from larger to smaller and the second row alternating from smaller to larger. In phase 2, we tested entrance height following the same design. We placed huts in each row about 1.5 m apart from one another and rows were 0.3 m apart from each other (Fig. 3). The water velocity within the artificial raceway is similar to that found in the Blue River release site and also that reported by Bodinof et al. (2012). The water velocity varied by inflow sections to simulate the variations in natural systems and the water velocity is generally higher upstream than downstream (Fig. 3). We introduced all Hellbenders at the top of the artificial raceway in each phase.

Data collection.—We checked all huts at 0900 on Mondays, Wednesdays, and Fridays, and conducted the experiment from 13 April to 16 October 2020. Checks were during daylight hours because Hellbenders are more active at night, decreasing the likelihood of being inside huts. We removed the lid for each hut and recorded the number of individuals in each hut, each individual PIT-tag identification, and the number of individuals not in huts during checks. We netted and minimally handled all individuals, and we released them back into their selected hut. We recorded the weight of each individual before and after each phase of the experiment. On Tuesdays, Thursdays, and Saturdays, we fed Hellbenders frozen Lake Smelt (*Hypomesus olidus*) individually with tongs to avoid interfering with data collection. We recorded water quality parameters (dissolved oxygen, pH, ammonia [NH_3], nitrate [NO_3-], and nitrite [NO_2-]) levels weekly. We used a syphon to spot-clean the raceway at least once weekly, or as needed, but did not disturb the huts during cleaning.

Statistical analysis.—We examined group-level occurrences of Hellbenders in huts to test whether they occurred in huts with different cavity volumes (phase 1) or huts with different entrance heights (phase 2) more often. At the group-level, we counted the number of Hellbenders in each hut per day of the experiment. First, we examined temporal changes in occurrences of Hellbenders in huts by plotting raw number and proportion of Hellbenders by day throughout the experiment. We then used Generalized Linear Mixed Models (GLMMs) to test whether more Hellbenders used small or large volume huts (phase 1) or hut entrances (phase 2) throughout the study and if this depended on the day of experiment (R package nlme; Pinheiro et al. 2017). We tested for effects on number and proportion of Hellbenders separately (two GLMMs per phase). We included a random effect for day to account for repeated measures of Hellbenders among days. To determine whether the effect of hut volume or entrance heights varied across days separately for number and proportion models, we compared between additive and interactive models using Likelihood Ratio Tests (LRTs; R package lmtest; Hothorn et al. 2019).

For the individual-level in both phases, we tested for selection of different hut volumes or entrance heights, for distance of huts downstream in the raceway (0.8, 2.3, 3.7, 5.4, 6.9, or 8.4 m), and for position of huts in the raceway (left or right side) using the Manly Resource Selection Function for Design II (Manly et al. 2002). We summed the number of occurrences of each Hellbender in each category throughout the experiment. We assumed that Hellbenders selected for hut characteristics independent of other Hellbenders because Hellbenders are not frequently reported to be found occupying the same shelter rock in the wild. We analyzed selection data in R package adehabitatHS (Manly et al. 2002; Calenge 2016). We tested the null hypothesis of selection in proportion to availability and rejected the null hypothesis if animals used habitats more (preference) or less (avoidance) compared to habitat availability (Manly et al. 2002; Calenge 2016).

We calculated selection ratios with 95% confidence intervals to identify preference (ratio greater than one) or avoidance (ratio less than one). We verified results of our individual-level analyses in phases 1 and 2 by re-running each analysis 10 times (size, position, and distance in phase 1 and 2; 60 total) using random subsets with 50% of the full dataset each time. Results from using all individual-level data were qualitatively similar to results from using any of the 10 random subsets of the individual-level data. We calculated the number of Hellbenders per day, per hut, and converted to percentages of huts with zero, one, and more than one individual per hut per day (mean percentage ± one standard deviation). We conducted all analyses and investigated assumptions of all analyses using program R v4.0.2 (R Core Team 2019).

Results

Phase 1 experiment.—We detected 89.4% (95% confidence interval [CI] = 87.7–91.0%) of Hellbenders in huts on any given day throughout the experiment. Only six Hellbenders (12.8%) occupied a single hut, but most occupied a mode of three huts (range, 1–6). At the group-level, the number and proportion of Hellbenders occurring in small volume huts significantly increased throughout (Hut size × Day; Table 1). Occurrence in different-sized huts depended on day for number and proportion of Hellbenders (i.e., support for interactive models from LRTs; χ^2 = 31.57 and 19.25, df = 2 and 2, respectively, both $P < 0.001$). Hellbenders occurred in small and large volume huts equally at the beginning of the phase (number and proportion); however, by the end, 70% of Hellbenders occurred in small volume huts and only 30% occurred in large volume huts (Fig. 4). Based on estimates from GLMMs, 2.68× more Hellbenders (number and proportion; 95% CI = 2.43–3.03 and 2.45–2.99×, respectively) occurred in small volume huts compared to large volume huts at the end (Table 1). At the individual-level, Hellbenders selected huts based on size (χ^2 = 890, df = 47, $P < 0.001$), position (χ^2 = 1,015, df = 47, $P < 0.001$), and distance (χ^2 = 3,361, df = 235, $P < 0.001$) downstream in the raceway. Hellbenders moderately

Table 1. Summary statistics for Generalized Linear Mixed Models assessing group-level number and proportion of Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) occurring in juvenile huts of different sizes throughout phase 1. Variables Hut size: Small was the reference level compared to Hut size: Large and Hut size: Small × Day is the interaction term. The abbreviations SE = standard error and df = degrees of freedom.

Variable	Estimate	SE	df	*t*	*P*
Number					
Intercept	20.864	1.020	27	20.46	< 0.001
Hut size: Small	1.255	1.442	27	0.87	0.392
Day	-0.142	0.027	27	-5.30	< 0.001
Hut size: Small × Day	0.281	0.038	27	7.42	< 0.001
Proportion					
Intercept	0.487	0.022	27	22.46	< 0.001
Hut size: Small	0.027	0.031	27	0.87	0.392
Day	-0.003	0.001	27	-5.82	< 0.001
Hut size: Small × Day	0.007	0.001	27	8.23	< 0.001

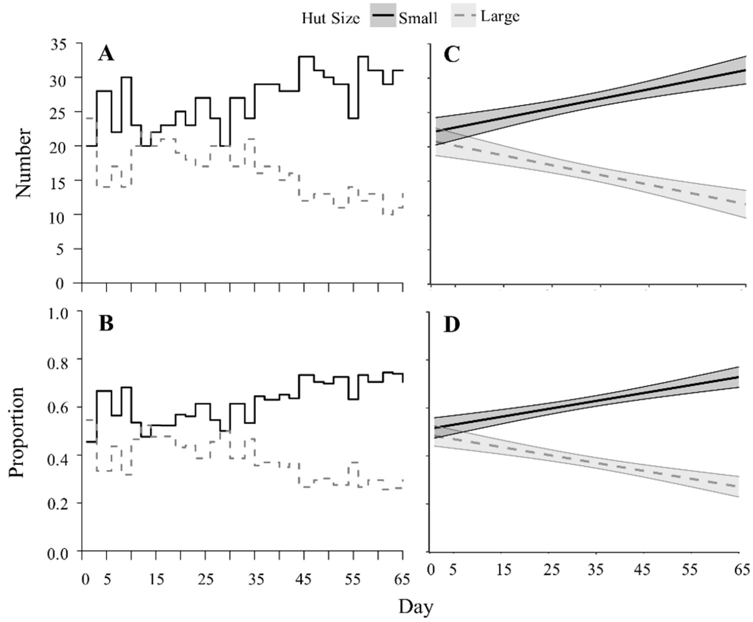

Figure 4. In phase 1, number (A and C) and proportion (B and D) of Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) occurring in small huts (solid lines) increased above those occurring in large huts (dashed lines) throughout phase 1. Panels A and B display raw data and panels C and D are mean, model-estimated number and proportion of Hellbenders with 95% confidence intervals.

preferred small volume huts (selection ratio 1.24, 95% CI = 1.00–1.48) and avoided large volume huts (0.75, 0.51–0.99; Fig. 5). Additionally, Hellbenders preferred huts on the right side of the raceway (1.13, 0.86–1.40) but the confidence interval slightly overlapped a selection ratio of 1.0, which indicates weak or no preference for huts on the right side. Hellbenders also weakly avoided huts on the left side of the raceway (0.86, 0.59–1.13; Fig. 5). Hellbenders strongly preferred huts that were farthest downstream in the raceway (8.4 m; 2.56, 1.58–3.53) and avoided (ratio < 1.0) or showed no selection (ratio overlaps one) for all other huts further upstream in the raceway (Fig. 5). We found that 18.1% ± 8.2% of huts were unoccupied, 33.3% ± 8.1% of huts were occupied by a single individual, and 48.6% ± 7.6% of huts were occupied by more than one individual on any given day.

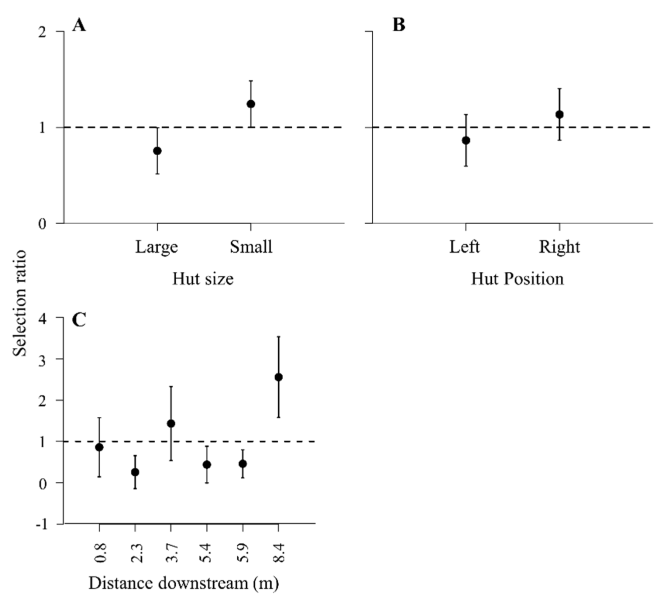

Figure 5. In phase 1, Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) selected for small huts (A), huts that were on the right side of the raceway (B), and huts that were farthest downstream in the raceway (8.4 m; C).

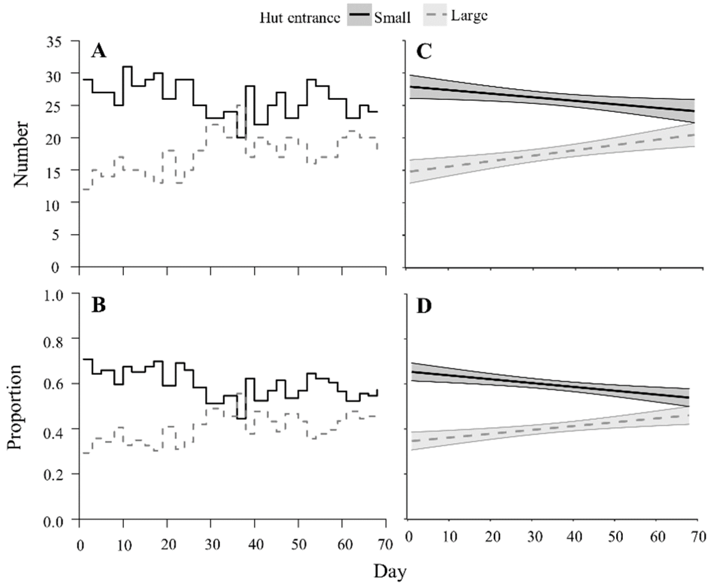

Figure 6. In phase 2, number (A and C) and proportion (B and D) of Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) occurring in huts with small entrances (solid lines) was higher than those occurring in huts with large entrances throughout phase 2. Those occurring in huts with small entrances, however, decreased throughout phase 2 and vice versa for huts with large entrances. Panels A and B display raw data and panels C and D are mean, model-estimated number and proportion of Hellbenders with 95% confidence intervals.

Phase 2 experiment.—We detected 92.8% (95% CI 91.8–93.9%) of Hellbenders in huts on any given day. Sixteen Hellbenders (34.8%) occupied only a single hut throughout phase 2, but most Hellbenders occupied a mode of two huts (range, 1–5). At the group-level, the number and proportion of Hellbenders in huts with small entrances was higher than those with large entrances but decreased throughout the phase (Hut size × Day; Table 2). Occurrence of Hellbenders in huts with small and large entrances depended on day (χ^2 = 6.05 and 6.94, df = 2 and 2, P = 0.048 and < 0.031, respectively). Seventy percent of Hellbenders occurred in huts with small entrances at the beginning of the phase, but only 57% occurred in huts with small entrances at the end (Fig 6). Compared to huts with large entrances, about 1.88× more (95% CI = 1.79–2.00) Hellbenders occurred in huts with small entrances at start of the phase; however, only about 1.17× more (95% CI = 1.16–1.19) occurred in huts with small entrances at the end (Table 2). Similar to the individual-level in phase 1, Hellbenders selected huts based on entrance height (χ^2 = 1,152, df = 46, P < 0.001), position (χ^2 = 1,173, df = 46, P < 0.001), and distance (χ^2 = 4,098, df = 230, P < 0.001) downstream in the raceway. Huts with small entrances were weakly preferred (selection ratio 1.19, 95% CI = 0.92–1.46) and those with large entrances were weakly avoided (0.81, 0.54–1.07; Fig. 7). Hellbenders strongly preferred huts that were farthest downstream (3.00, 1.95–4.04) and avoided or showed no selection for all other huts upstream in the raceway (Fig. 7); however, Hellbenders moderately preferred huts on the left side of the raceway (1.25, 0.99–1.51) and weakly avoided those on the right in this phase (0.75, 0.48–1.01; Fig. 7). We found that 3% ± 4.5% of huts were unoccupied, 56.7% ± 8.5% of huts were occupied by a single individual, and 31.9% ± 6.8% of huts were occupied by more than one individual on any given day.

Table 2. Summary statistics for Generalized Linear Mixed Models assessing group-level number and proportion of Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) occurring in juvenile huts with different entrance heights throughout phase 2. Variables Hut entrance: Small was the reference level compared to Hut entrance: Large and Hut entrance: Small × Day is the interaction term. The abbreviations SE = standard error and df = degrees of freedom.

Variable	Estimate	SE	df	*t*	*P*
Number					
Intercept	14.718	0.911	28	16.15	< 0.001
Hut entrance: Small	13.215	1.289	28	10.25	< 0.001
Day	0.084	0.023	28	3.71	< 0.001
Hut entrance: Small × Day	-0.141	0.032	28	-4.36	< 0.001
Proportion					
Intercept	0.345	0.020	28	17.05	< 0.001
Hut entrance: Small	0.311	0.029	28	10.88	< 0.001
Day	0.002	0.001	28	3.37	0.002
Hut entrance: Small × Day	-0.003	0.001	28	-4.77	< 0.001

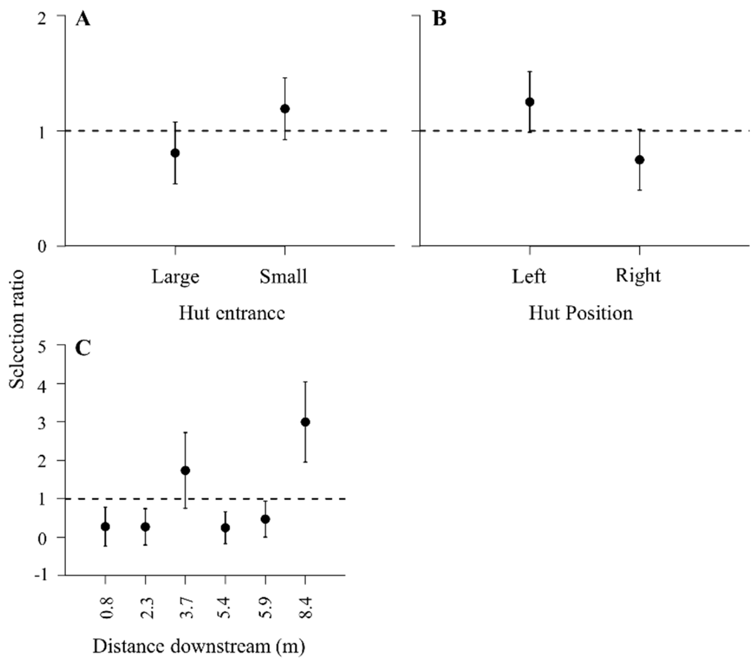

Figure 7. In phase 2, Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) selected for huts with small entrances (A), huts that were on the left side of the raceway (B), and huts that were farthest downstream in the raceway (8.4 m; C).

Discussion

Hellbenders selected for smaller cavity volumes throughout the experiment. For phase 1, we found Hellbenders in both cavity volumes equally at the beginning of the experiment but primarily occupied smaller cavity volume huts by day 15. Captive-reared Hellbenders previously released in the Blue River showed similar usage of shelter rocks that were approximately half the size of those used by wild adults (McCallen et al. 2018). Moreover, in phase 1, approximately 87% of individuals occupied more than one hut and moved between several huts. With evidence of interchange, the majority of Hellbenders experienced attributes of different huts in both phases but still had a strong preference toward smaller cavity volume. Social interactions could influence selection of habitats either by displacing one another or by aggregating in certain habitats, and it is difficult to truly determine group preference for habitat characteristics. Single animal trials would more accurately represent preference for hut characteristics, but our system setup precluded this study design. Our results, however, strongly indicate preference for small cavity volumes and the current study design emulates the conditions of an actual Hellbender release. Further research will need to focus on hut deployment in the wild to determine if deploying multiple small volume huts near release sites could increase shelter use and survival for captive-reared juveniles released in the Blue River in Indiana.

In phase 2, initially we found Hellbenders in huts with smaller entrances but, by the end, they occurred in huts with different entrance heights at nearly equal proportions. While Hellbenders displayed a slight preference for smaller entrances at the end of the study, use was trending towards equilibrium, and it is likely that had the experiment run longer, use would have been approximately equal. Therefore, we suggest that juveniles have no strong preference between the entrance heights used in our study, but our study included no consequences for entrance size choice, and in a natural setting, larger entrances could result in increased vulnerability to predators or exposure to environmental variations. It is possible that the initial selection of smaller entrance sizes is instinctive, but the lack of consequence led to individuals spreading evenly throughout shelters despite entrance size. Furthermore, *in situ* entrance size preference could vary depending on local conditions (e.g., water velocity, sediment deposition, and turbulence). Previous attempts to deploy artificial shelters in Virginia, USA, were hindered because sedimentation blocked entrances and shelters became unavailable to Hellbenders (Button et al. 2020b). It is unclear how the entrance heights of huts in the current study will resist sedimentation once deployed in the river, and continuous monitoring of huts to ensure accessibility will be important.

Ontogenetic shifts could also explain the preference Hellbenders showed towards huts placed farthest downstream in both phases. In the Blue River, McCallen et al. (2018) found that resident wild adult Hellbenders were more likely to disperse upstream and translocated wild adults were found equally likely to disperse either upstream or downstream; however, all translocated captive-reared juveniles dispersed downstream. Exposing our Hellbenders to a large artificial stream raceway designed to simulate a natural stream setting, with variation in stream velocities throughout at water inflow sections might have encouraged our juveniles to move downstream into the lower huts where water velocity is lower. Further research including adults Hellbenders would be necessary to determine ontogenetic differences in velocity preferences between life stages.

Although Hellbenders preferred huts downstream, they moved among several different huts, both upstream and downstream, throughout each phase of the experiment. We alternated hut types throughout the stream to ensure stream flow did not confound preference. Furthermore, our results could differ if selection of habitats by Hellbenders is not independent, but we ran our analysis with 10 random subsets, which suggests that our results are robust to non-independence if it occurred in our dataset. Even with hut interchange, Hellbenders might have ultimately ended downstream due to a preference for lower water velocity. Further experimentation would be beneficial in understanding Hellbender stream velocity preference.

Juvenile huts may prove to be critical in dispersal zones between release sites as juvenile Hellbenders establish home ranges. Providing shelter in low-quality habitat dispersal zones between high-quality habitat release sites could potentially increase captive-reared juvenile survival rates by providing shelter during flood events, preventing individuals from being forcefully carried downstream, and protecting against predation (McCallen et al. 2018). In the Little River in Tennessee, Hecht et al. (2019) observed differences in shelter size between different Hellbender age classes suggesting an ontogenetic shift in habitat use. Our study observed only one age class hatched in 2015. Different age classes might prefer huts of different sizes or design. Also, different hut designs might be more or less suitable for varying habitat types (i.e., pool, riffle, run) and different hydrologic regimes. Future studies testing shelter-size preference with varying age classes, hut designs, and in different habitat types within a controlled environment could be beneficial for understanding developmental shifts and overall hut preference.

Increasing survival of captive-reared Hellbenders is essential to restoring populations in Indiana and throughout the historic range of the species. We determined hut preferences of juvenile Hellbenders and this information will hopefully increase survival of juveniles in the future when shelters are deployed in the Blue River. Our research aimed to conserve the Hellbender population in Indiana and help Hellbenders move closer to becoming a self-sustaining population within the Blue River. The findings of our study could be useful to aid juvenile Hellbender populations throughout their range. If the use of our huts result in increased post-release survival, their use should become a standard practice in future Hellbender translocation programs across the country.

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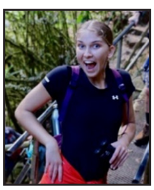

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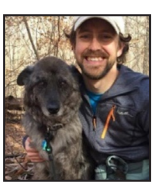

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