Rearing captive eastern hellbenders (*Cryptobranchus a. alleganiensis*) with moving water improves swim performance

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ABSTRACT

Translocations often use captive-reared animals to help bolster or re-establish wild populations. However, captive environments are highly dissimilar from wild conditions and may deprive animals of experiences that promote normal development. Captive-rearing and translocation efforts are underway for eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*). Yet, hellbenders reared in aquaria that lack stimuli often make long-distance downstream movements following release, perhaps because of their naïveté to riverine environments. We altered standard captive techniques and reared juvenile hellbenders with (treatment) and without (control) water current for 18 months. We quantified morphological plasticity and swim performance as a function of rearing environment to assess the value of environmental enrichment in hellbender head-start programs. We compared broad-scale growth rates for mass, snout-vent length, and total length, but found no difference between treatment and control hellbenders (mass difference = 0.1 g/month, P = 0.596; snout-vent length difference = 0.01 cm/month, P = 0.360; total length difference = 0.01 cm/month, P = 0.533). We also examined fine-scale tail morphology measurements and found treatment individuals developed more shallow tails that grew 49% slower than control individuals during the rearing period (mean difference = 0.86 mm/month, P = 0.017). We interpret this as evidence of either energy expenditure or phenotypic plasticity as more streamlined tail forms are found in lotic systems. Moreover, we found water current to be positively associated with hellbenders' swimming ability. After three swim trials, treatment hellbenders were 46% quicker in their swim time (P = 0.033), required 29% fewer upstream attempts (P = 0.012), and were 60% less likely to need manual motivation to make it to an upstream tile hide (P = 0.010). Moreover, treatment hellbenders tended to improve these responses linearly through time (P = 0.016) compared to control individuals that showed no improvement across the three trials (P = 0.075). Together, our data suggest that the addition of water current to hellbender rearing environments does not have any detrimental impact on hellbender body morphology, but rather, acclimates hellbenders to moving water and improved their ability to reach upstream refugia. We advocate incorporating water velocities, representative of natural conditions, into hellbender captive-rearing programs. Rearing animals with semi-natural conditions in captivity may better prepare animals for and potentially improve the success of future translocations. This advancement to standard rearing techniques may positively influence the preservation of wild hellbender populations throughout the nation.

1. Introduction

Translocations and reintroductions are popular conservation strategies designed to bolster or re-establish wild populations. Although popular, fewer than half of translocations are classified as successful (Griffith et al., 1989; Fischer and Lindenmayer, 2000; Germano and Bishop, 2009). Many translocations release young age classes as they are easier to transport, can be collected in greater numbers, and will be less likely to demonstrate homing tendencies following release (Germano and Bishop, 2009). However, animals in their early life stages are at the greatest risk of mortality (Haskell et al., 1996). Therefore, focus has been directed toward head-start programs where animals are reared in captivity during their most vulnerable stage to reduce rates of mortality.

Head-starting provides safety and ample amounts of food to ensure rapid growth, but can still be inadequate in preparing individuals for natural living conditions (Alberts, 2007). Artificial rearing environments are highly dissimilar from wild conditions and can deprive animals of experiences and stimuli that promote the development of necessary behavioral skills and morphological responses to environmental

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stressors (McPhee and Carlstead, 1990; Hard et al., 2000; Kelley et al., 2005). Captive-reared animals may lack developed muscle tone, endurance, and sprint speeds making them morphologically distinct from wild populations (Young and Cech, 1993; Ward and Hilwig, 2004; Connolly and Cree, 2008). Moreover, individuals may adopt behaviors that are advantageous in captivity, but maladaptive in the wild, further hindering translocation success (e.g., Stoinski et al., 2003).

To combat morphological and behavioral deficiencies, some head-starting programs have incorporated environmental enrichment techniques (see Bashaw et al., 2016; Biggins et al., 1998; Salvanes et al., 2013). Environmental enrichment aims to mimic nature by introducing captive animals to live prey, incorporating more natural refugia, structural complexity, and microhabitat characteristics, and training them to adopt appropriate survival skills prior to release (Biggins et al., 1998; Ward and Hilwig, 2004; Alberts, 2007). For example, black-footed ferrets (*Mustela nigripes*) raised with live prey are more successful at locating and killing prey compared to untrained conspecifics (Vargas and Anderson, 1999). Captive Siberian polecats (*Mustela eversmani*) have heightened antipredator responses when trained with predator models and aversive stimuli (Miller et al., 1990). Also, hatchery-reared fishes reared with moving water have increased growth, weight, and muscle mass, and after only 50 days of exercise conditioning show similar trait values and performance to wild individuals (Young and Cech, 1993, 1994; Ward and Hilwig, 2004). Therefore, animals exposed to simulated natural conditions in captivity may be better equipped (i.e., behaviorally, morphologically, and physiologically) for introductions into the wild (Berejikian et al., 2000).

Head-start programs for eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) have been established to combat precipitous population declines. Captive-rearing efforts for hellbenders are effective at facilitating growth, but hellbenders are reared from egg to juvenile in aquarium tanks that lack stimuli. Subsequently, static aquarium environments may leave juvenile hellbenders naïve to natural conditions (e.g., riverine water velocities, predator cues, or complex habitat). Stamps and Swaisgood (2007) argue that captive conditions could be important determinants of post-release movement as translocated animals are more likely to leave release sites if they don't encounter cues, physical features, or microhabitat similar to their captive environment. Some hellbender rearing facilities incorporate substrate and structural complexity more similar to natural environments, but environmental enrichment has never been used in preparation for hellbender translocations.

It is unknown whether translocation failure is correlated with the head-starting environment, but previous hellbender augmentations have had variable success (17–72% survival over six months; Bodinof et al., 2012; Boerner, 2014; Kraus et al., 2017). Hellbenders are fairly sedentary throughout the year, usually moving infrequently and only relatively short distances between shelter rocks (27.5 m), if at all (Burgmeier et al., 2011). Yet, 70–100% of captive-reared individuals released to the wild show downstream dispersal, 40–60% making abrupt long-distance movements permanently away from core habitat (50–1800 m), and many becoming completely lost following flood events (Bodinof, 2010; Kraus et al., 2017). Long-distance, post-release movements are particularly adverse to translocations, and are positively correlated with mortality as a large proportion of deaths can be attributed to dispersal away from high quality habitat (Moehrenschlager and Macdonald, 2003; Stamps and Swaisgood, 2007; Bodinof, 2010). Moreover, unintentional or intentional long-distance movements can negatively influence survival rates as individuals expend excessive amounts of energy and become more susceptible to predation while outside of refugia (Ward and Hilwig, 2004; Bodinof, 2010).

Amphibians exhibit considerable phenotypic plasticity, expressing changes in behavior, morphology, life history, or physiology in response to predators, competitors, or their rearing environment (Wilbur, 1987; Relyea and Werner, 2000; Relyea and Hoverman, 2003).

Exposing juvenile hellbenders to natural water current in a captive setting may induce behavioral plasticity such as swimming skills, navigation techniques, and endurance. Moreover, hellbenders naturally have an oar-like tail, which they rely on to orient their body and propel them during bouts of sprint swimming. Although their primary mode of locomotion is through lateral undulation, exposure to elevated water velocity may lead to more swimming, which may induce plastic changes such as a wider and more utilitarian tail against water current. A variety of enrichment techniques (e.g., structural complexity, variability in prey, and natural vegetation) have been found to be effective in reversing maladaptive phenotypes and may be beneficial for hellbenders' development (Biggins et al., 1999; Ahlbeck and Holliland, 2012; Hyvärinen and Rodewald, 2013). Therefore, a combination of captive-rearing efforts that introduce semi-natural conditions, as well as provide safety and food to juveniles may be the most viable solution to acclimate and prepare hellbenders for release into the wild.

We investigated whether juvenile hellbenders exhibit morphological plasticity in their body and tail and examined the swim performance of hellbenders in simulated, flood-like conditions as a function of rearing environments with and without elevated water velocity. We predicted that hellbenders reared with moving water would grow faster, would have longer and deeper tails, and would have better upstream swimming ability against flood-like water velocity. This project has the potential to advance current rearing techniques for eastern hellbenders, provide valuable information for captive facilities, and positively influence future translocation efforts.

2. Methods

2.1. Study species and environmental enrichment

Hellbenders are North America's largest salamander, growing up to 74 cm in length (Petranka, 1998). They reside in cool, fast-flowing rivers and are distributed throughout the Midwest and southeastern areas of the United States (Mayasich and Phillips, 2003; Petranka, 1998). Although some healthy populations occur in parts of North Carolina, Virginia, West Virginia, and Tennessee (usually associated with the Appalachian mountain range and preserved forests), hellbender populations have suffered drastic population declines over the past few decades (Mayasich and Phillips, 2003). Threats such as sedimentation, water pollution, and human misconceptions have reduced available habitat, lead to disease or illness, and extirpated local populations (Mayasich and Phillips, 2003; Wheeler et al., 2003). In Indiana, hellbenders are only found in a single river system and a recent population viability analysis reported complete extirpation by 2035 if no management action was taken (Burgmeier et al., 2011; Unger et al., 2013). Reproduction was last documented in Indiana in 2015, but there has been no evidence of hellbender recruitment for the past 20 years (Kern, 1984). This suggests geriatric hellbenders (living up to 30 years of age) are the remaining stronghold to the wild population. The population viability analysis found that positive changes in early life-stage survival and the number of reproductively viable females (above the age of six) could increase population growth (Unger et al., 2013). Therefore, efforts to improve juvenile survival are critical to the persistence of Indiana's remaining wild population.

We created captive environments with and without water current at Purdue University's Aquaculture Research Laboratory in West Lafayette, Indiana, USA. We constructed a system with six polyethylene raceways (Pentair Filtration sump – S207095, Pentair Aquatic Ecosystems, Apopka, FL, USA: $1.4 \times 0.6 \times 0.6$ m, L × W × H): three treatment raceways with elevated water velocity (0.2–0.3 m/sec) and three controls with slow flow (0–0.05 m/sec). We designed our raceway system as a part flow-through, part recirculating system to remove solid waste, sterilize water, and reduce iron levels. We maintained velocities between 0.2–0.3 m/sec in the treatment raceways because hellbenders are naturally found in riffles and runs varying in flow rate from

0.16–0.34 m/sec (Bodinof et al., 2012). Control raceways were maintained between 0 and 0.05 m/sec to mimic standard rearing conditions that lack moving water. We collected a single clutch of hellbender eggs from southern Indiana, USA in 2013. We reared these individuals in 132 liter aquarium tanks with simple PVC and tile hides and no added substrate for two years. We randomly selected 120, two-year olds for this experiment, all of which weighed on average 30.6 g (SE ± 0.46 g). We recorded spot patterns from the dorsum and tail of each of these hellbenders for unique identification and randomly assigned 20 to each treatment or control raceway (n = 120).

We fed hellbenders twice weekly with equal amounts of black worms (*Lumbriculus variegatus*) or crayfish (*Orconectes rusticus*). We standardized the amount of food between treatments at each feeding event. Diets varied monthly depending on availability of worms and crayfish, but all raceways received the same type of food and at comparable amounts of biomass: 35–40 g of worms and 20–25 crayfish per raceway. We always made sure to place worms behind the inflowing water pipe to prevent flushing them out of the raceways with water current. We also provided multiple shelter rocks (30 × 50 cm flagstone rocks) and assorted gravel substrate in raceways.

2.2. Morphologic comparisons

We reared all hellbenders in raceways for 18 months, from May 2015 to December 2016, and collected morphological measurements and lateral photographs at the beginning and end of the enrichment period. Hellbenders are slow-growing species, yet we predicted 18 months would be enough time to observe changes in size. Moreover, it would allow us to rear animals until they were nearly four years old, which is a standard age to release hellbenders back into the wild (Kraus et al., 2017). We measured broad-scale snout-vent length (SVL; cm), total length (TL; cm), and mass (g), being sure to identify each individual during morphologic measurements. We also placed hellbenders into a V-shaped, glass holding device to take a standardized lateral photograph to measure fine-scale tail length and depth (Olympus camera – SP-57OUZ, Olympus, Center Valley, PA, USA).

We calculated growth rates (end-beginning/18 months) for all broad-scale body morphology measurements (SVL, TL, and mass) and fine-scale tail morphology measurements (tail length and depth). We used ImageJ software to measure tail length, from vent to tail tip, and tail depth, at the deepest point between the dorsal and ventral sides of the tail, with lateral photographs of all the hellbenders (Schneider et al., 2012). We compared broad-scale growth rates between treatment and control individuals using univariate linear regressions and included 'raceway' in our model to account for multiple hellbenders within an individual raceway. We conducted univariate ANOVAs for fine-scale growth and included raceway and mass. We included the growth rate for mass as a covariate in fine-scale comparisons to control for size variation across individuals (Hoverman and Relyea, 2012). We report mean differences and estimated growth rates. We ran all analyses in Program R version 3.3.2 and assigned an alpha level of 0.05 (R Core Team, 2014).

2.3. Swim performance trials

To evaluate hellbenders' ability to navigate and endure high flow events, we tested the swim performance of all hellbenders (n = 119 because of a previous mortality) in an artificial stream following 18 months of environmental enrichment. We used a 2.4 × 0.3 × 0.3 m (L × W × H) polycarbonate trough with water depth of 25 cm, a flow meter and tile hide placed at the upstream end, and a screen to catch hellbenders at the downstream end (Fig. 1; similar to Bestgen et al., 2010). We did not include gravel substrate in the artificial stream because we wanted to mimic bedrock sections of a natural river and motivate individuals to swim upstream rather than remain stationary. We constructed our stream system so that a centrifugal pump
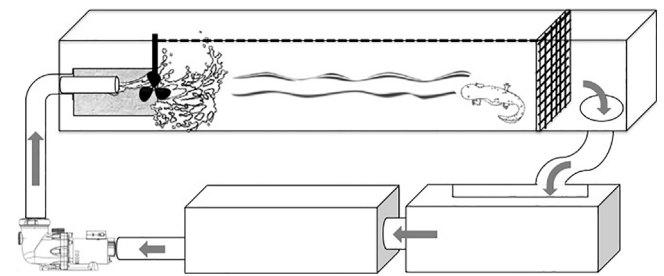

Fig. 1. Schematic of the artificial stream used for swim performance trials. Water entered into the stream channel through the pipe on the far left side, which was placed above the upstream tile hide. Water passed by a flow meter, dissipated downstream, emptied into two adjoining sumps, and then recirculated by the attached water pump. The hellbenders were placed at the bottom of the stream channel for each trial and observed for a maximum of ten minutes, using the black notches (1 notch = ~5 cm) to measure initial upstream movement during that time.

(Sequence® 1000 Series – Model 6100SEQ23, MDM Incorporated, Colorado Springs, CO, USA) pulled water from two large polyethylene sumps, pushed water past the flow meter and down the length of the stream channel, and then recirculated water through the stream back into the holding sumps. This allowed for maximized flow to be continually pumped into the channel.

Prior to our experimental trials, we introduced each of the 119 hellbenders to the stream channel individually and allowed them eight minutes to freely explore and acclimate to the system at low water velocities (~0.5 m/sec, Ward et al., 2003). These acclimations occurred once, 7–9 days prior to trials. An acclimation period is common in performance trials as this allows individuals to become oriented to the testing environment and, in this case, the presence of the upstream tile hide (Bestgen et al., 2010). Following the acclimation period, we evaluated hellbenders' swim performance across three trials, with two rest days between each event (Adolph and Pickering, 2008). We designed our study with three trial events in order to observe possible changes and improvement through time (Adolph and Hardin, 2007; Irschick and Meyers, 2007; Kupferberg et al., 2011).

We concentrated on the ability of hellbenders to endure flood-like velocity conditions and move upstream against current; therefore, we opened the gate valve to achieve and maintain a maximum velocity around 1.46 m/sec (nearly five times faster than the velocity in treatment raceways). At the beginning of each trial period, we identified individuals by spot patterns and then placed a single hellbender at the bottom of the artificial stream. We recorded time to start moving, initial distance moved upstream before the hellbender fell back or turned around, the number of upstream attempts, and swim time (i.e., time when hellbenders started moving until the end of the trial). Time ended if hellbenders successfully completed the trial by making it under the upstream tile hide or if ten minutes passed. If a hellbender did not move or complete the trial after five minutes we motivated them with soft taps on their tail and repeatedly turned them to face upstream after failed attempts (Shaffer et al., 1991). Once the trial was completed or ten minutes had passed, we returned them to their designated raceways.

We compared time to start moving, initial distance moved upstream, swim time, number of upstream attempts, likelihood of requiring motivation, and probability of successfully completing the trial and making it to the tile hide, as a function of rearing environment. Due to non-normal data, we conducted generalized linear mixed regressions with penalized quasi-likelihood, used a Gaussian distribution, and log link (R package 'MASS') for continuous (i.e., measures of time and distance) response variables. We used generalized linear regressions with appropriate link functions for Poisson (i.e., number of attempts) and binomial (i.e., used motivation and successfully made it to tile hide) responses, as these did not meet the assumptions of penalized quasi-likelihood. We tested full models that included treatment, trial,

and an interaction between treatment and trial as our fixed effects. We included individual nested within raceway as our random effects to account for repeated measures. We compared the treatment effect at each trial and changes across trials through time. We back-transformed estimates to report means and mean differences. We ran all analyses in Program R version 3.3.2 and assigned an alpha level of 0.05 (R Core Team, 2014).

3. Results

3.1. Morphologic comparisons

All hellbenders increased in size throughout the experiment, without differences in broad-scale growth rates for mass, snout-vent length, or total length between treatments (mass difference = 0.1 g/month, t value = −0.58, df = 1, P = 0.596; snout-vent length difference = 0.01 cm/month, t value = 1.03, df = 1, P = 0.360; total length difference = 0.01 cm/month, t value = 0.68, df = 1, P = 0.533). There were no differences among these measurements, likely because they were highly correlated with one another (correlation values > 0.75, P value < 0.001). We found no differences in fine-scale tail length or depth at the beginning of the experiment (t value = 0.453, df = 1, P = 0.651 and t value = −1.495, df = 1, P = 0.136, respectively). Moreover, changes in tail length were similar among treatment and control hellbenders (mean difference = 1.6 mm/month, t value = −2.29, df = 1, P = 0.084; Fig. 2a). However, fine-scale growth rates in tail depth were significantly different in treatment compared to control hellbenders (mean difference = 0.86 mm/month, t value = −3.93, df = 1, P = 0.017). More specifically, treatment hellbenders had 49% slower change in their tail depth through time (95% CIs = 24–60%), with significantly more shallow tails at the end of the enrichment period compared to control individuals (t value = −3.19, df = 1, P = 0.033; Fig. 2b).
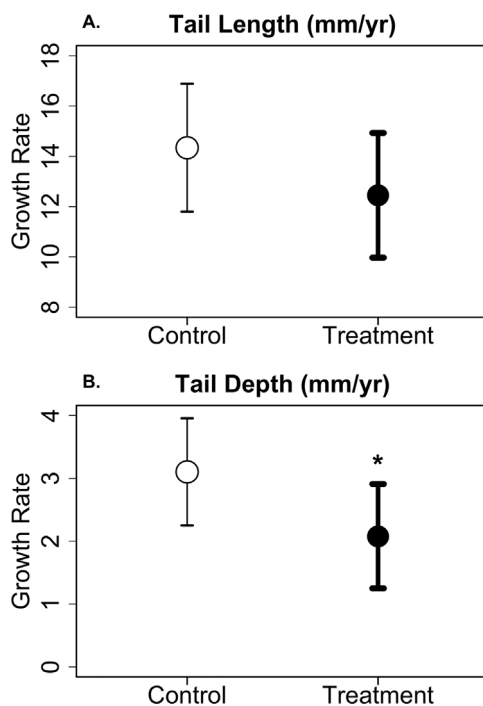

Fig. 2. Fine-scale tail morphology growth rates of hellbenders (*Cryptobranchus a. alleganiensis*) from control and environmental enrichment treatment raceways. Growth rate estimates for tail length (A.) and tail depth (B.) are units per year, presented with 95% confidence intervals. Individuals from the treatment raceways have 33% slower change in tail depth through time compared to those from the control raceways. An asterisk denotes significant differences at an alpha level of 0.05.

Table 1
Model estimates from six swim performance responses measured for control and treatment hellbenders. Test statistics are *t* values for swim time, initial distance, and time to move. Test statistics are *z* values for number of attempts, probability of needing motivation, and probability of completing trial. All trials were completed in November 2016 with 119 eastern hellbenders (*Cryptobranchus a. alleganiensis*); bolded values indicate significant differences.

Response		Control	Treatment	Test statistic	P
Swim time (sec)	Trial 1	198.53	155.58	−1.47	0.145
	2	145.28	121.75	−0.81	0.419
	3	149.6	80.49	−2.16	**0.033**
Number of attempts	Trial 1	3.47	3.77	0.08	0.535
	2	3.52	2.89	−0.19	0.161
	3	3.73	2.63	−2.51	**0.012**
Probability of needing motivation	Trial 1	0.465	0.333	−0.55	0.158
	2	0.327	0.264	−0.74	0.462
	3	0.362	0.146	−2.59	**0.010**
Initial distance (cm)	Trial 1	145.52	146.25	0.11	0.913
	2	160.77	157.58	−0.48	0.635
	3	161.93	154.68	−1.08	0.282
Time to move (sec)	Trial 1	60.35	70.07	0.64	0.521
	2	53.02	39.86	−0.86	0.394
	3	54.33	47.97	−0.42	0.673
Probability of completing the trial	Trial 1	0.95	0.92	−0.63	0.528
	2	0.95	1.00	0.01	0.999
	3	0.88	0.98	1.51	0.130

3.2. Swim performance trials

Treatment and control hellbenders were similar in their initial distance, time to move, and probability of completing each trial (Table 1). However, by the third trial conditioned hellbenders were 46% faster in their swim time (mean difference = 69 s, P = 0.033), required 29% fewer upstream attempts to reach the upstream hide (mean difference = 1.1 attempts, P = 0.012), and were 60% less likely to need motivation to move (mean difference = 0.22, Table 1, Fig. 3). Moreover, there were linear changes through time, such that treatment hellbenders became 20% faster at each trial (95% CI = 7–30%, mean estimate = 37.5 s per trial, t value = −2.94, df = 1, P = 0.004). Treatment hellbenders also reduced their number of attempts by 18% (95% CI = 9–25%, mean estimate = 0.57 attempts per trial, t value = −3.82, df = 1, P < 0.001) and their probability of needing motivation by 41% through time (95% CI = 35–48%, mean estimate = 0.094 per trial, t value = −2.39, df = 1, P = 0.017). Alternatively, control hellbenders showed no improvement in swim time (mean estimate = 24.5 s per trial, t value = −1.79, df = 1, P = 0.075), reduction in the probability of needing motivation (mean estimate = 0.05 per trial, t value = −1.14, df = 1, P = 0.255), or any significant change in number of upstream attempts across trials (mean estimate = −0.13 attempts per trial, t value = 0.81, df = 1, P = 0.417).

4. Discussion

We found no detrimental effects of environmental enrichment on hellbender growth. Rearing fish with moving water can increase growth among some salmonids; but unlike fish, hellbenders do not continually swim and generally only come out of refugia to forage (see Davison, 1997). Instead, hellbenders' growth rates increased at comparable rates between treatments, which is similar to exercise conditioned goldfish (*Carassius auratus*), chub (*Leuciscus cephalus*), nace (*Chondrostoma nasus*), and branded wrasse (*Notolabrus fucicola*) that show no change in growth compared to conspecifics reared without moving water (Davison and Goldspink, 1978; Lackner et al., 1988; Hinterleitner et al., 1992; Sanger, 1992; Davison, 1994). Although body morphology was similar between treatments, we found hellbenders reared with elevated water velocity exhibited more shallow tails compared to control hellbenders. Most vertebrates expend more energy while swimming and
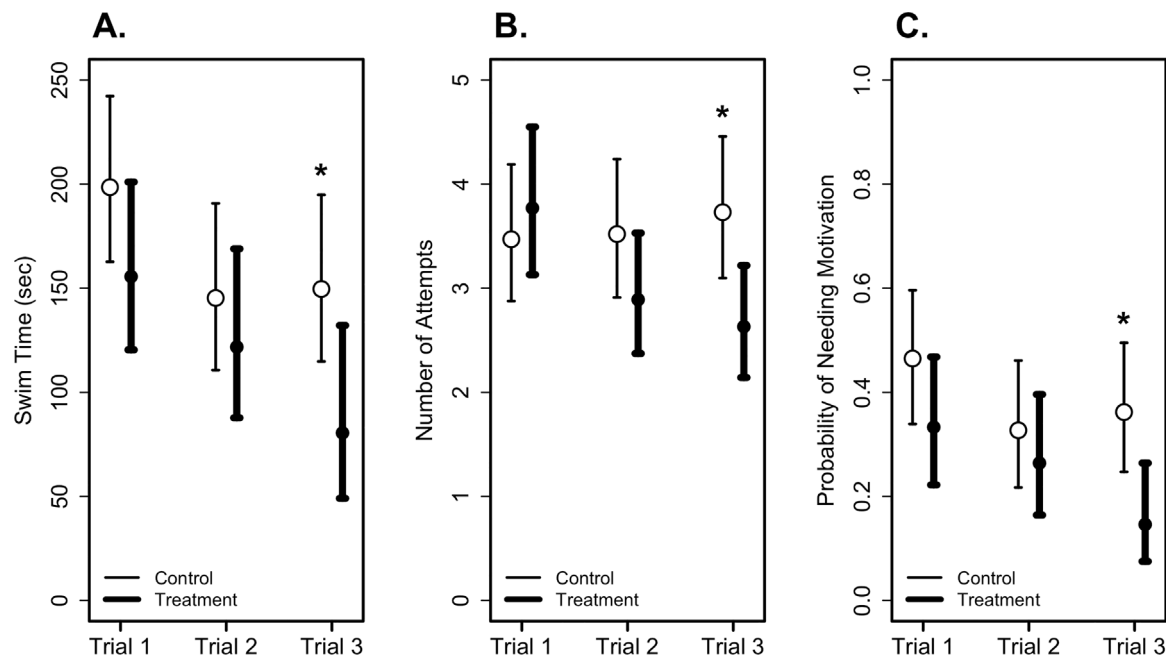
Fig. 3. Differences in swim time (A.), number attempts (B.), and probability of needing motivation (C.) between eastern hellbenders (*Cryptobranchus a. alleganiensis*) reared without (control) and reared with (treatment) elevated water velocities, across three swim performance trials. By the third trial, treatment hellbenders were quicker to complete the trial, required fewer attempts to reach an upstream tile hide, and were less likely to need manual motivation to move. Model estimates with 95% confidence intervals are presented; an asterisk denotes significant differences at an alpha level of 0.05.

perhaps more shallow tails was a result of energy use rather than energy storage. Fish reared at higher velocities have reduced whole body fat content and salamanders, including hellbenders, commonly store energy and deposit fat in their tails (Ogata and Oku, 2000; Wells, 2007; Personal Communication). Elevated energy expenditure might have reduced the available stores and subsequently affected average tail depth.

Alternatively, shallow tails are evidence of phenotypic plasticity as animals reared in heterogeneous environments have a greater chance for plasticity to occur. The presence of water current my have induced a more streamlined tail, which is common among salamanders naturally residing in lotic systems (Wells, 2007; Haad et al., 2011). Larvae that live in ponds typically have large tail fins that extend up the back, whereas larvae that live in brooks and rivers tend to have small, shallow tail fins that end at the body (Wells, 2007). A shallow tail is thought to reduce drag, similar to streamlined body forms. For example, shallow-bodied crucian carp (*Carassius carassius*) reared without predatory pike (*Esox lucius*) experience 32% less drag while swimming compared to deep-bodied conspecifics reared with pike (Brönmark and Miner, 1992). Regardless, of the mechanism inducing more shallow tails, treatment individuals had improved swimming ability, which suggests deeper tails are not necessarily indicative of increased maneuverability. Often, body condition or body mass are used to assess locomotor traits, but size may be an unreliable metric of locomotion potential (Pérez-Tris et al., 2004; Vervust et al., 2008). Instead, swim performance is more likely a function of experience. Exercised and unexercised spikedace (*Meda fulgida*) have comparable length measurements, but have up to 40% improvement in swim performance following swim training (Ward and Hilwig, 2004). Rearing efforts are usually tailored to magnify size and expedite growth, but rather, should be focused on experience and performance skills.

As seen in our results, hellbenders rearing with water current demonstrated improved swim performance. By the third swim trial, treatment hellbenders had a faster swim time, were less likely to need motivation, and they required fewer attempts to reach the upstream tile hide compared to control individuals. Previous exposure to water current may have improved endurance and allowed individuals to become

better acclimated to riverine conditions. Similarly, fish reared in tanks with elevated water velocity have faster swim speeds, swim further, and are less susceptible to fatigue than fish reared with lower current (Pearson et al., 1990; Young and Cech, 1993; Ward and Hilwig, 2004). Fishes that have been exercise conditioned with moving water are thought to have greater stamina than unconditioned individuals (Hammond and Hickman, 1966; Lackner et al., 1988; Ward and Hilwig, 2004). Treatment hellbenders in our experiment may have perceived water current as a novel experience, therefore needing motivation to move, been less physically prepared, becoming fatigued after two trials, and subsequently, were less likely to reach the upstream tile hide by the third trial.

Not only were treatment hellbenders quicker to complete the swim challenge by the third swim trial, but they showed significant improvement in their swimming skills, navigation, and motivation through time. Conditioning, training, or imprinting techniques to improve performance have been successful among many animals, including amphibians (Burghardt, 2013). For example, salamanders (*Salamandra salamandra*) can recognize and preferentially choose prey objects after foraging experience, and tadpoles (*Rana lessonae* and *Rana esculenta*) show advantageous predator avoidance behaviors after previous exposure to predator stimuli (Luthardt-Laimer, 1983; Semlitsch and Reyer, 1992). There is also support across taxa that variable environments aid in developing flexible and plastic behaviors (Braithwaite and Salvanes, 2005). Hellbenders have an innate ability to swim, as all individuals were able to make it to the upstream tile hide at least once; however, we observed behavioral plasticity among some of the treatment individuals that were more successful and quicker to make it upstream. These individuals swam along the bottom of the artificial stream rather than at the water surface and required fewer attempts to reach the tile hide. This behavior might be advantageous following release into the wild to reduce the likelihood of hellbenders being swept downstream and the potential for predator attacks at the water surface.

Our study provides evidence that environmental enrichment can better acclimate individuals to riverine-like environments and corroborates evidence that pre-release training can improve skills such as

swimming, navigating current, and finding refuge. Previous hellbender translocations have documented abrupt long-distance-downstream dispersal and lowered survival when individuals disperse more than 50 m from their core release habitat (Bodinof et al., 2012). Animals that move more become prone to exhaustion, being swept downstream during flood events, or occupying low-quality habitat – ultimately leading to translocation failure. Stocked razorback suckers (*Xyrauchen texanus*) have high downstream movement when they are initially released into river systems, likely because rearing stream-adapted fishes in standing water adversely impacts their ability to survive in lotic environments (Brooks, 1986; Ward and Hilwig, 2004). Hellbender salamanders are found in fast-flowing, well-oxygenated rivers; therefore, it makes intuitive sense that these animals would be at a disadvantage when reared in aquarium tanks. Our findings suggest that advancing captive-rearing techniques to include environmental enrichment may have positive effects on translocation success.

We found that rearing two-year old hellbenders with moving water for 18 months was successful in producing advantageous changes in swim performance. Hellbenders are usually released into the wild between the ages of three and six; therefore, future work should aim to better understand the importance of starting age, duration, and intensity of water velocity when using environmental enrichment techniques prior to release efforts (Bodinof et al., 2012; Kraus et al., 2017). Furthermore, additional work should investigate how rearing hellbenders with elevated water velocities influences survival, habitat use, and movement following their translocation into the wild. If hellbenders maintain upstream swimming skills once released, are able to withstand elevated and variable flow regimes, and are capable of returning to refuge objects, they may have a higher probability of survival even if they are unintentionally moved downstream. Incorporating other semi-natural conditions beyond water velocity (e.g., predator cues and riverine microbiota) into captivity to mimic animals' release environments may have profound effects on translocation success and the future of imperiled populations in the wild.

5. Conclusions

Environmental enrichment has been used effectively with a variety of vertebrate animals, but has never been used as a method to improve the success of hellbender translocation projects (Ward and Hilwig, 2004). This study provides substantial evidence that incorporating riverine-like water velocities into the captive-rearing environment improves hellbender swim performance. Moreover, this acclimation to moving water came without morphological trade-offs in weight or length growth rates. This information has strong implications for current hellbender rearing facilities, but also for endangered, imperiled, or at risk species that have struggled to transition from captive to wild environments. If animals reared in more natural environments prior to release prove to do better in the wild than captive individuals that are untrained and inexperienced, then altering standard rearing techniques to include semi-natural conditions can positively influence translocation efforts and the preservation of wild populations in the future.

Conflicts of interest

None.

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