



# 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 (Moehrensclager 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 Eco-systems, Apopka, FL, USA:  $1.4 \times 0.6 \times 0.6$  m,  $L \times W \times H$ ): 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

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