North American Journal of Aquaculture

ISSN: 1522-2055 print / 1548-8454 online
DOI: 10.1002/naaq.10065

SPECIAL SECTION: CAPTIVE PROPAGATION OF IMPERILED SPECIES

Propagation of Eastern Hellbenders *Cryptobranchus alleganiensis alleganiensis* in a Recirculating Aquaculture System at Shepherd of the Hills State Fish Hatchery

James A. Civiello*
Missouri Department of Conservation, Shepherd of the Hills Fish Hatchery, Branson, Missouri 65616, USA

Timothy J. Bruce
University of Idaho, Department of Fish and Wildlife Sciences, Moscow, Idaho 84843, USA

Stuart J. Brisco
Missouri Department of Conservation, Shepherd of the Hills Fish Hatchery, Branson, Missouri 65616, USA

Jeffrey T. Briggler
Missouri Department of Conservation, Resource Science Division, Jefferson City, Missouri 65109, USA

Abstract
The hellbender *Cryptobranchus alleganiensis* is an endangered aquatic salamander inhabiting coldwater streams within the Ozark Highlands of Missouri. Over the past few decades, there has been a severe decline in the populations of the two subspecies of hellbender in this region—the Eastern hellbender *C. a. alleganiensis* and the Ozark hellbender *C. a. bishopi*—accompanied by little to no recruitment of young age-classes; projections now call for negative long-term population growth for both subspecies. Captive propagation is thus considered essential for the long-term recovery of this species in Missouri, along with addressing the causes for its decline in the wild. Since 2007, Shepherd of the Hills Fish Hatchery has been the primary rearing facility in Missouri for the early life stages of Eastern hellbenders. This facility has focused on hatching fertilized eggs collected from the wild and rearing animals for repatriation into their natal streams. This paper describes the recirculating aquaculture system used to rear Eastern hellbenders, the culture techniques that have been developed, and some of the results.

The hellbender *Cryptobranchus alleganiensis* is a large aquatic salamander inhabiting moderate to swift-flowing rivers and streams of the eastern United States, with two recognized subspecies inhabiting the Ozark Highlands of Missouri and Arkansas. One of these, the Eastern hellbender *C. a. alleganiensis*, has been known to occur in 16 states ranging from central Missouri to southern New York and southwest to northern Alabama and northeastern Mississippi (Petranka 1998). In Missouri, the Eastern hellbender has been known to inhabit the Meramec River, Big River, Gasconade River, Osage Fork of the Gasconade River, Big Piney River, and Niangua River. At this time, Eastern hellbender populations are known to occur in the Gasconade, Big Piney, and Niangua rivers, with some individuals potentially still being present in the Meramec River, Big River, and Osage Fork of the Gasconade River (J. T. Briggler, unpublished data).

Native Missouri populations of Ozark hellbenders *C. a. bishopi* and Eastern hellbenders have declined by more than 70% over the past 40 years due to degraded water

*Corresponding author: james.civiello@mdc.mo.gov
Received May 2, 2018; accepted August 26, 2018

quality, habitat loss, disease, predation, illegal collection for the pet trade, and habitat disturbance (Wheeler et al. 2003; Briggler et al. 2007, 2010). There has also been a prominent shift in hellbender age structure toward mature individuals, while young age-classes have been virtually absent (Wheeler et al. 2003; Briggler, unpublished data). As a result of these drastic population declines, the Ozark and Eastern hellbenders were listed as critically imperiled and endangered in Missouri in 2003, and in 2011 the Ozark hellbender was listed as endangered under the federal Endangered Species Act (USFWS 2011).

Population modeling assessments have indicated a high risk of extinction (above 96%) over the next 70 years for both subspecies in Missouri (Briggler et al. 2007). The severe decline of hellbenders in Missouri, accompanied by the lack of young age-class recruitment, has shifted the conservation efforts toward the establishment of successful captive-rearing systems and culture techniques to ensure long-term recovery.

Currently, there are two facilities devoted to long-term captive propagation efforts in Missouri. The Ron Goellner Center for Hellbender Conservation WildCare Institute at the Saint Louis Zoo is primarily focused on Ozark hellbender propagation (Briggler et al. 2012a), while the Missouri Department of Conservation's Shepherd of the Hills Fish Hatchery (SHFH) in Branson is primarily focused on rearing Eastern hellbenders for conservation (Briggler et al. 2014).

Little published information on the captive rearing of hellbenders is available, and culture techniques vary among rearing facilities (Ettling et al. 2013, 2017; Unger and Mathis 2013). Over a 10-year period, SHFH staff have developed a recirculating aquaculture system (RAS) to incubate and hatch Eastern and Ozark hellbender eggs collected from the wild and to grow Eastern hellbenders to 50 g during a 3-year culture period (Briggler et al. 2014). This paper describes the RAS system that we have developed, the techniques that have been developed to raise hellbenders, their growth and survival in captivity, and the lessons that we have learned.

METHODS

Rearing facility.— Shepherd of the Hills Fish Hatchery is located below Table Rock Dam in Branson, Missouri. The hatchery sits on 62.72 ha, which includes access to Lake Taneycomo for anglers. Construction of the hatchery coincided with that of the dam in 1957. The primary purpose of the hatchery is to supply Lake Taneycomo with a yearly commitment of 475,000 27-cm (11 in) Rainbow Trout *Oncorhynchus mykiss*.

Recirculating aquaculture system design and specifications.— One RAS for hellbender propagation includes the following: a Smart Model 025120 UV Sterilizer (Pentair Aquatic, Apopka, Florida), a TECO TK 115-V 1000 Aquarium Chiller (Pentair Aquatic) to keep the water temperature at 16°C, a Premier 200 wet/dry trickle filter (Pro Clear Aquatic Systems, Jacksonville, Florida), a 290-W W70HD pump (Pentair Aquatic), four linear aluminum rearing tanks and one polyethylene TP110 416-L rectangular sump tank (all from Pentair Aquatic; Figure 1). The entire RAS contains a total volume of 568 L of water, inclusive of the tanks, sumps, UV filter, and plumbing lines. The four linear aluminum rearing tanks are 304 cm long × 35 cm wide × 18 cm deep, and the removable PVC standpipe allows for an average water depth of 6.35 cm (tank volume is approximately 70 L). The magnetic drive pump (Figure 2) circulates water from the sump to a height of 1.5 m and distributes it to a PVC (2.54 cm) plumbing manifold that distributing it to the four linear tanks, providing a flow of 28 L/min to each tank. This flow rate provides approximately 20 exchanges of water per hour through each tank. Lexan polycarbonate sheet lids are hinged along the 304-cm length of each tank, and latching mechanisms have been installed for security (Figure 3). A sterilized well water supply flows through the four rearing tanks and drains into a Plexiglas trickle filter, providing both mechanical and biological filtration. Blue Bonded filter pads (Pentair Aquatic) provide mechanical filtration, as water will flow by gravity through the biomedia to a 416-L sump tank, completing the water flow path of the closed system (Figure 4). Estimated costs for these components are provided in Table 1.

Water source.— The main water supply for the hatchery is Table Rock Lake, and the hatchery water intake is located approximately 43 m below the water's surface.

FIGURE 1. Recirculating aquaculture systems for Eastern hellbender propagation at Shepherd of the Hills State Fish Hatchery (SHFH). The large gray tanks serve as support structures for the insulated aluminum hellbender rearing tanks with their lids, filtration units, and blue sumps. This area of flow-through fish production has been converted into the RAS for hellbender production.

FIGURE 2. Photograph showing the 290-W W70D pump that comprises part of each RAS. Each system has one such pump and obtains water from two sumps. Water is diverted into the chilling unit and UV sterilizer before being distributed to the four linear tanks.

FIGURE 3. Linear tanks fitted with Lexan lids for biosecurity. There are three sections per tank, each having a hinged and hasped area for a padlock.

Hatchery water has an annual temperature range of 4–11°C, and hatchery facilities utilize well water as a mixing source (if needed) to control rearing water temperatures. With this combination of well and lake water, a temperature of approximately 10°C can be maintained throughout the year.

Water treatment.— The hellbender rearing tank systems were initially set up as a single-pass system supplied by well water into linear tanks. Early in the development of these rearing techniques, amphibian chytrid fungus *Batrachochytrium dendrobatidis* was detected within the rearing tanks and the open well water supply was suspected as the

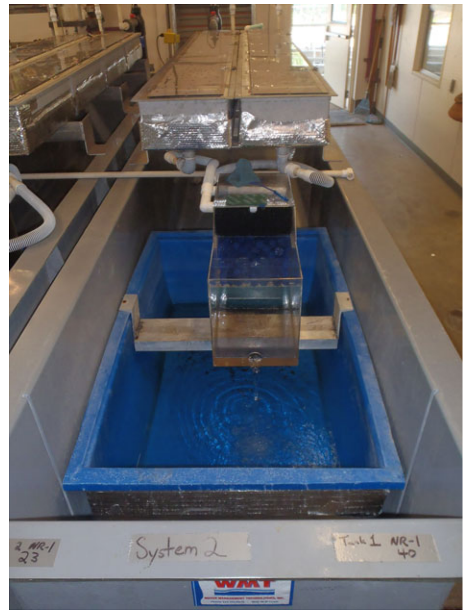

FIGURE 4. Each RAS has 4 linear tanks, 2 trickle filters, and 2 sumps. This allows for a single trickle filter to be removed for egg incubation in a vertical incubation system.

route of transmission. Amphibian chytrid fungus is a highly contagious pathogen prevalent in amphibians that causes their skin to thicken or grow a fungus which affects the animal's ability to breathe (Berger 2001). This particular fungus has been known to occur within wild hellbender populations in Missouri (Briggler et al. 2008; Bodinof et al. 2011) and likely negatively impacts (i.e., via direct mortality, increased stress, suppressed immune responses, secondary bacterial and fungal infections, etc.) the species in confined, captive conditions (Junge 2012). The suspected open water source may have also led to chytrid outbreaks in two separate year-classes of captive Eastern hellbenders (2007 and 2011). Due to the repeated disease outbreaks and the mortality of animals, increased biosecurity measures were implemented by designing and constructing a closed RAS. In 2014, hatchery staff implemented water sterilization via heating and added lids to the system, as described in detail below. Heat sterilization was done to reduce the threat of amphibian chytrid fungus as well as other potentially harmful aquatic pathogens (Berger 2001; Webb et al. 2007). The 2013 and 2015 year-classes have since screened as negative for the pathogen.

Within this hellbender rearing system, the security of the animals has been of upmost importance. SHFH staff have previously observed the potential for these animals to escape the rearing tanks. Further, the public has shown great interest in these SHFH hellbenders, and there has been a need to reduce the potential for spreading pathogens through direct contact and limit stress for these animals during allocated and permitted tours. The

TABLE 1. Estimated costs to acquire and construct a single recirculating aquaculture system for hellbender propagation.

Item	Manufacturer	Model or item description	Approximate cost ($US)
Aluminum linear tanks (4)	Rose Metal Company	304 × 35 × 18 cm	1,000
Magnetic drive pump (1)	Pentair Aquatic Ecosystems	W70HD (290 W)	300
Wet/dry trickle filter	Pro Clear Aquatic	Premier 200	290
Polyethylene sump tank (1)	Pentair Aquatic Ecosystems	TP110	250
High output UV sterilizer (1)	Pentair Aquatic Ecosystems	Smart 025120 (E120S)	850
0.5-hp chiller unit (1)	Pentair Aquatic Ecosystems	Teco TK (SCTK3000)	1,800
Lexan polycarbonate (sheet)	Grainger Industrial Supply	1ETT7	150
PVC plumbing and fittings	Local source	1½ in	100
All items			4,740

installation of hinged and hasped Lexan polycarbonate sheet covers (Grainger Industrial Supply, Miami) equipped with padlocks on the rearing tanks has improved tank security. These locked covers reduce the potential for the transmission of diseases derived from wild amphibians found inside and near the hatchery as well as possible human transfer of pathogens (SHFH is a large fish production facility with high visitor throughput). The objective of this new technique was to develop a closed system with treated water and secure tanks that would increase biosecurity and the survival of hellbenders to release size while in captivity. Since the implementation of these system modifications and biosecurity upgrades, the disease has been better managed and there has been a substantial increase in the overall health status of these salamanders. To further prevent the spread of disease to these animals, SHFH has implemented an encompassing biosecurity plan which is regularly updated.

A system of heat-treating the well water used in the rearing system was specifically needed to sterilize it against the amphibian chytrid fungus. The sterilization design consists of four 208-L plastic barrels (repurposed and cleaned formalin barrels from fish production) interconnected with 5.08-cm PVC plumbing to allow circulation from barrel to barrel by means of a Pentair 290-W W70HD magnetic drive pump. A 1,800-W Easyplug LH18 resistance immersion heater (Pentair) with an Aqualogic thermostat (Aqualogic, San Diego, California) is used in one of the barrels to maintain a temperature of 37°C for 4 h (Johnson et al. 2003). After the appropriate heat exposure time has been achieved, the power to the heater is turned off. Water is circulated through the interconnected barrels, and the water temperature eventually returns to the ambient air temperature. This treated water (757 L) is used for a weekly 50% water change in the rearing system or small additions of water to compensate for evaporation. After every water change, API Stress Coat (Mars Fishcare, Chalfont, Pennsylvania) is added to the RAS at up to 1 mL per 38 L of tank rearing water. This therapeutic additive reduces stress, promotes healing and the regeneration of damaged tissue, and provides the hellbenders a synthetic slime coating on their epithelia.

Culture techniques.— In September or early October, we observe fertilized egg clutches in the wild but have typically left them in their natural habitat for most of the embryo development to increase hatching success. Entire or partial clutches are mostly collected when the egg stage of development is ≥18 (see Smith 1912 for the stages of egg development in hellbenders) and transported to SHFH in a 19-L bucket equipped with a battery-powered aerator and covered with a dark towel to reduce the exposure of the eggs to light. The stream water and egg clutch are tempered to the captive-rearing system temperature in the bucket using treated well water from the sterilized water supply at SHFH. Eastern hellbenders are raised to the minimum release size of approximately 50 g weight and 20 cm total length in order to safely tag each individual with a passive integrated transponder (PIT) tag prior to release. We initiated the use of vertical incubation trays in 2007, and now these trays are commonly used for hellbender egg incubation in other captive-rearing programs (Briggler et al. 2014). Vertical incubation trays similar to the products manufactured for use in salmonid culture (Heath Tecna Corp., Kent, Washington) are utilized over the 14- to 30-d egg incubation period (Figure 5; the exact length of the period depends on the developmental stage of the clutch). A stack of 12 trays is located over the sump within the closed system until the eggs hatch. When an egg clutch is placed in a 30.48-cm × 40.64-cm vertical incubation tray, the strand of egg capsules is cut apart with scissors and tweezers and placed flat in a single layer to aid in daily inspection of the developing embryos and simplify enumeration (Figure 5). Approximately 200 eggs are placed on each tray. After the egg clutch has been cut apart, it is laid in a single layer inside the incubation tray and photographed; the yolks are manually counted from the printed image.

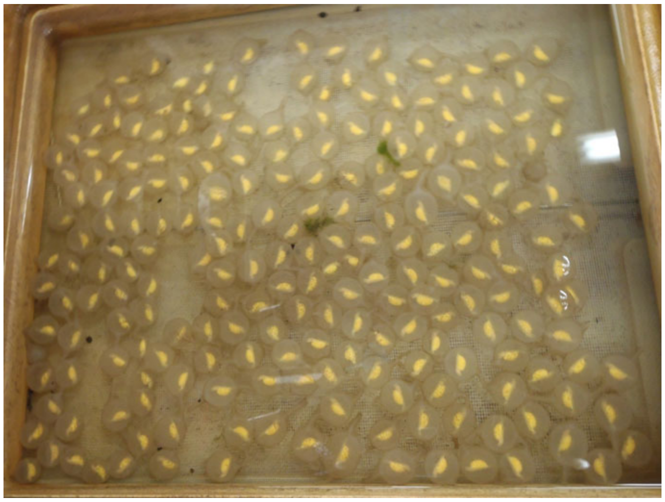

FIGURE 5. Developing Eastern hellbender embryos in a vertical incubation tray.

After hatching, the larvae are moved from the incubation trays to the aluminum linear tanks. Up to 200 larvae are placed in a 10,600-cm^2 area of tank bottom space (roughly 50 cm^2 of bottom space per animal). Each tank is provided with "hide rocks" or other hiding structures such as 20-cm × 20-cm floor tiles propped up with rocks or short lengths of PVC pipe 1.27 cm in diameter, which several animals can use for cover. A 1:2 ratio of open bottom to creek gravel is provided to simulate the natural Ozark stream environment and to enable the juvenile hellbenders to develop foraging behavior. Total length (TL) and weight data were recorded every 4–6 months until the salamanders were released.

The primary diet offered from the time that the yolk sac is absorbed until 1 year of age are isopods (*Caecidotea brevicauda*) and amphipods (*Gammarus pseudolimnaeus*). These aquatic invertebrates, which are abundant locally and serve as the primary food base for native fish species in many Ozark streams, are collected in a variety of sizes palatable to <1-year-old larval hellbenders from the fish hatchery water discharge outfalls to Lake Taneycomo. California blackworms *Lumbriculus variegatus* are purchased and provided to the developing animals. From 1 to 5 years of age, earthworms *Eisenia fetida*, thawed *Mysis* spp., brine shrimp *Artemia* spp., and freeze-dried krill *Euphausia* spp. are fed until the time of reintroduction at 3 years of age and/or a weight of approximately 50 g. Purchased or otherwise acquired nonfrozen foods must first be treated to eliminate or reduce the risk of disease or bacteria exposure to the hellbenders. Isopods, amphipods, and blackworms are rinsed with dechlorinated tap water to remove the original water sources and then soaked in a 50 ppt salt concentration for 5 min; the food is rinsed again before being weighed and rationed to the animals (Briggler et al. 2014). With respect to feeding amounts, an average tank (containing twenty-five 3-year-old animals) will consume approximately 600 g of these prey items per month. In an effort to track growth metrics and evaluate overall hellbender health in the rearing systems, detailed tank records of feeding rates and water quality are maintained and archived in a database. Feeding behavior is observed daily and adjustments are made to avoid wasting food.

Water quality parameters are monitored weekly and recorded. The following parameters are maintained: pH, 7.75–8.0; nitrate, <10 mg/L; nitrite, <0.2 mg/L; nitrogen ammonia, 0.02–0.05 mg/L; and dissolved oxygen, >90% saturation. Corrections are made when any parameter is outside of its accepted range, but weekly monitoring and tank cleaning generally alleviate the need for adjustments. However, dissolved oxygen levels are kept below the saturation level for any given water temperature for the gilled, larval stages via a reduction in air stones or air emittance through the stones. If the animals obtain air bubbles within their cephalic cavity, they may lose buoyancy and float in the tank water.

Disease surveillance and mitigation.— To accompany the hellbender rearing program, a disease management strategy has been implemented to ensure optimal health under conservative aquaculture conditions. Diagnostic testing for amphibian chytrid fungus is conducted every 6–8 months or when pathological symptoms are observed. To perform this surveillance, sterile swabs of hellbender epithelium are sent to a commercial lab for analysis via molecular testing. This action was incorporated into the rearing program to reduce the potential for a pathogen to spread throughout the RASs in the event of a disease outbreak. Also, prior to release of the animals into the wild, they are tested for amphibian chytrid fungus, as described below.

Other pathogens encountered on hellbenders at SHFH are variants of *Aeromonas* spp. (specifically, *A. hydrophila*, *A. cavaie*, and *A. veronii*) and *Pseudomonas* spp. Further, specific bacterial pathogens and subsequent tissue maladies have previously been investigated in Ozark hellbenders (Nickerson et al. 2011). These bacteria are commonly found in the rearing systems, but they must be kept in check to prevent diseases. In the past, there have been cases in which these bacterial pathogens externally adhered to juvenile hellbenders. Such infections are defined by reddish inflammation on the digits with subsequent "degloving" (sloughing of the external epithelium) and lesions on the digits. This particular bacterial infection is easily treated with topical antibiotics under the supervision of a veterinarian. Saprolegniasis (water mold) is controlled by treatment with copper sulfate at 500 mg/L for 2 min once daily for 5 d. In an effort to detect these pathogens when clinical signs become apparent, bacterial culture and genetic testing have been implemented. In such cases, swabs of hellbender digits and epithelia are streaked onto tryptic soy agar and incubated at 22°C for several days for bacterial identification. In the event of any suspected disease issues, it is important to coordinate efforts with a team of aquatic health collaborators, especially those with experience with amphibians and their diseases. In addition, the treatment of one pathogen (e.g., bacterial infection) can lead to additional health issues (e.g., fungal infection); therefore, treatment dosages must be measured to ensure that the protective skin microbiota are maintained in order to promote recovery and enhance the health of these animals.

Preparation for release.— Prior to the release of captive-reared individuals, the following measures are taken to increase the success of augmentations and/or reintroductions of Eastern hellbenders into the wild: A representative sample (>50 individuals from each tank system) are typically tested for amphibian chytrid fungus five to six times using nonlethal skin swabs for PCR assays. If the individuals in a system have not previously tested positive for the pathogen, they are subjected to two testing periods approximately 6 months prior to release. If they have tested positive for the fungus at any point, they will be treated via heat treatment as described in Briggler et al. (2012b) and Junge (2012) to kill the fungus. Treated animals must have a minimum of three consecutive negative tests prior to release. These measures ensure that no chytrid-positive animals are released into the wild. A general health screening based on physical appearance (abnormalities, weight loss, skin texture, etc.) is also conducted prior to release.

Future identification of released animals is essential to evaluate the success of augmentation in the wild. Released hellbenders are marked with a PIT tag (Biomark, Boise, Idaho) inserted in the tail region that allows for individual identification. Individuals are PIT-tagged when they are approximately 50 g, and captive-reared individuals are released in the same watershed from which broodstock or eggs were collected. The preferred time of release for these captive-reared animals is generally May–August, but the actual time is dependent on the particular considerations at each site (intensive recreational use, water levels, potential increased predation, etc.). Captive-reared individuals are transported in aerated and temperature-controlled containers to the release sites.

RESULTS AND DISCUSSION

Over the 10 years that SHFH has been involved with the Ozark and Eastern hellbender recovery efforts, we have received 22 egg clutches from six different streams representing both subspecies and have achieved a 72% hatching rate from fertilized eggs to larvae (Table 2). Hatchling success has ranged from 33% to 98%; the number of larvae was greater when eggs were collected late in embryo development (Table 2). Since 2007, 3,886 hellbender larvae have been hatched and reared at SHFH (Table 2). After hatching and being started on feed, over 1,400 Ozark hellbender larvae from the 2007, 2010, and 2011 year-classes were transferred to the St. Louis Zoo propagation facilities for rearing. To date, a total of 641 Eastern hellbenders that were hatched and reared at SHFH have been released into three watersheds (the Big Piney, Gasconade, and Niangua rivers) in which the eggs

TABLE 2. Hatching results for Eastern and Ozark hellbender egg clutches collected from the wild and transferred to Shepherd of the Hills State Fish Hatchery (SHFH) from 2007 to 2015.

Year	River	No. fertilized eggs	No. hatched eggs	No. clutches	Estimated stage of development at collection[a]	Hatching success (%)
2007	Big Piney[b]	677	458	2	7	68
	North Fork[c]	127	61	2	2–3	48
2010	Eleven Point[c]	182	154	1	20	85
	North Fork[c]	102	34	1	2–3	33
2011	Niangua[b]	970	786	2	14	81
	Gasconade[b]	885	228	3	2–3	26
	Current[c]	143	138	1	20	97
	North Fork[c]	1,130	1,053	3	22	93
2013	Niangua[b]	380	176	1	17	46
	Gasconade[b]	149	135	1	18	91
2015	Niangua[b]	347	332	3	19–22	96
	Big Piney[b]	339	331	2	20–22	98
Total		5,431	3,886	22		72

[a]Stage of egg development as outlined in Smith (1912).
[b]Eastern hellbenders.
[c]Ozark hellbenders.

where originally collected. In 2010, 104 captive-reared young stemming from wild egg clutches collected in 2007 were released into the Big Piney River. This marked the first in-state release of captive-reared Eastern hellbenders in Missouri (Briggler et al. 2011). Additional releases occurred in 2012 (Big Piney [85] and Gasconade [2] rivers), 2013 (Niangua River [150]), and 2017 (Niangua [127] and Gasconade [8] rivers). The most recent releases occurred in 2018 (Niangua [99] and Big Piney [66] rivers). Subsequent surveys have captured several captive-reared individuals that appeared to be healthy in that they had grown. At this time, the animals released in 2010 and 2011 have shown signs of reproductive maturity, and it is hoped that they will contribute to increased reproductive output and subsequent recruitment.

At an average water temperature of 18°C, the 2007, 2011, and 2013 year-classes of Eastern hellbenders reached the minimum release size of approximately 50 g in 1,000 d (Figure 6). Over this period, annual feed costs were about $900.00.

Among the 2007, 2011, 2013, and 2015 year-classes of Eastern hellbenders, there was only one fungal diagnosis that required chemical treatment in the first two year-classes and only one bacterial diagnosis in the last two year-classes. Our experience has shown that hellbenders are most vulnerable to disease after 3 years of age.

Hellbender Morphometrics

Using morphometric data from the length and weight inventories of the animals, we derived relationships pertaining to predicted weight and length and growth rates. Recent studies have investigated the influence of rearing system conditions (such as water movement) on growth and overall body morphology (Kenison and Williams 2018). Thus, comparison of the metrics for specific systems found by these studies may provide insight into the optimal design of hellbender culture systems. Figure 7 shows the relationship between the number of days since hatch and TL. From 300 to 900 d, TL increased about two-fold (10.08 cm).

Figure 6 depicts the relationship between days from hatch and weight, while Figure 8 shows the relationship between total length and weight for the 12 hellbender clutches. As shown in Figure 6, the deviations in hellbender weights were notably smaller prior to the 600-d mark. From days 300 to 900, there was a 4–5-fold weight increase of 39.96 g. Estimated growth rates for hellbenders reared at SHFH were calculated by dividing the differences between the final and starting weights for each age-class by the number of days in the period and then multiplying by 100. Both TL and weight increased substantially within the first 30 d posthatch, but the estimated growth rates fell appreciably at approximately 1 year posthatch (Figure 9). The derived morphometric data were then used to establish appropriate rearing densities (Table 3). These data can be also used to adjust feeding rates throughout the rearing period. And in the future, hellbender health at SHFH may be evaluated by comparing the observed morphometric rates and estimated growth with the established standards at each sampling time.

Lessons Learned and Future Plans

The most important lesson that we have learned over the past 10 years is that it is imperative to employ a closed culture system for biosecurity control, followed by the need to have an optimal biosecurity plan and utilize sufficient water and feed sterilization procedures. The unique-looking, endangered hellbenders with which we work are native to Missouri, and our captive-rearing efforts have generated a lot of interest from the visiting public. Having locking lid covers has helped to prevent contamination of the closed culture systems, which are in close proximity to open fish culture tanks and are a natural attraction for humans, who are eager to see and handle

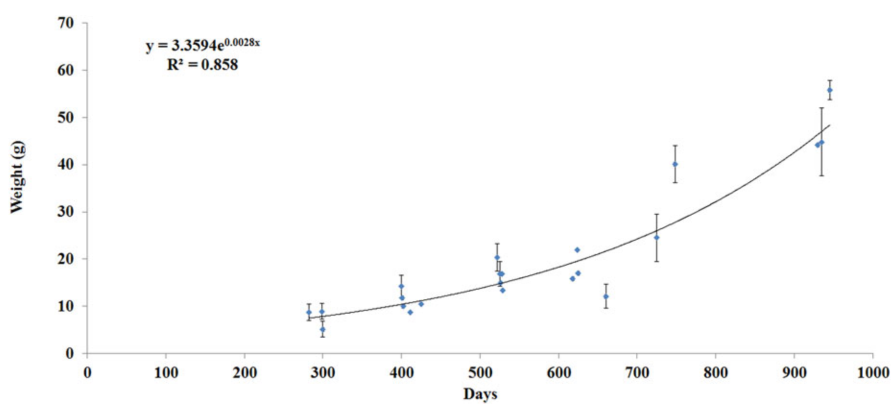

FIGURE 6. Estimated rate of increase in weight of Eastern hellbenders from approximately 300 d to 1,000 d. Data are from 12 egg clutches hatched at and stocked from SHFH from 2011 to 2017. Each data point represents multiple tank measurements recorded during a single sampling event on the same day that were then averaged; the error bars are SDs.

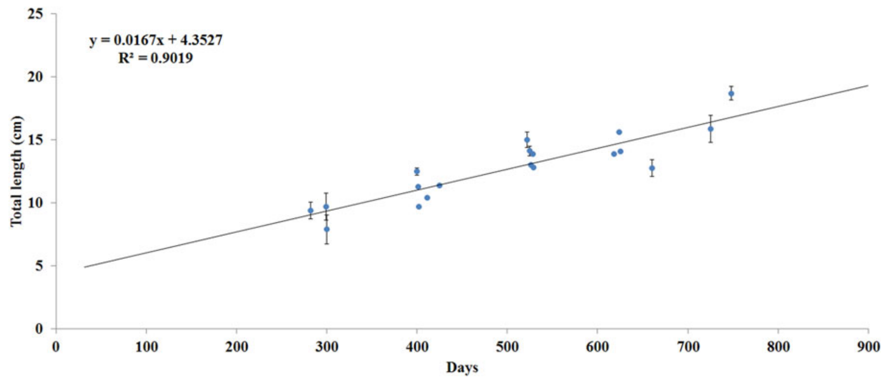

FIGURE 7. Estimated rate of increase in the length of Eastern hellbenders from hatch to release into the wild. The data are from 12 Eastern hellbender egg clutches hatched at and stocked from SHFH from 2011 to 2017. Each data point represents multiple measurements recorded during a single sampling event on the same day that were then averaged; the error bars are SDs. To minimize handling, initial count numbers were based on observed and hatched larvae. Count estimates were made at the time the hellbenders were moved from vertical incubation trays to the tank system. Lengths were recorded after 1 year and approximately every 6 months thereafter until the time of release.

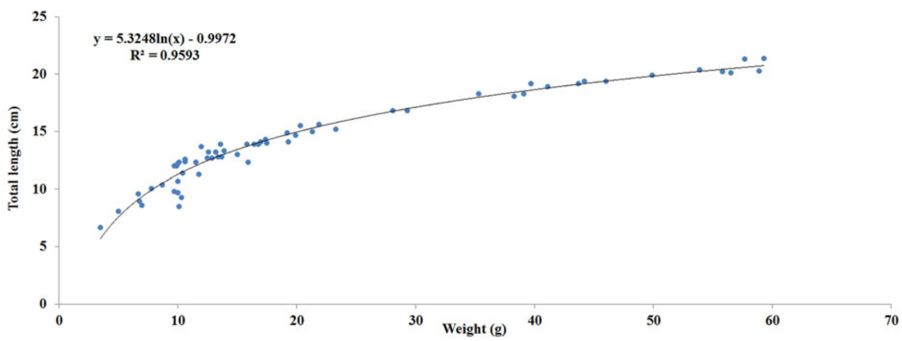

FIGURE 8. Estimated relationship between total length and weight for Eastern hellbenders from the 12 egg clutches described in Figure 6.

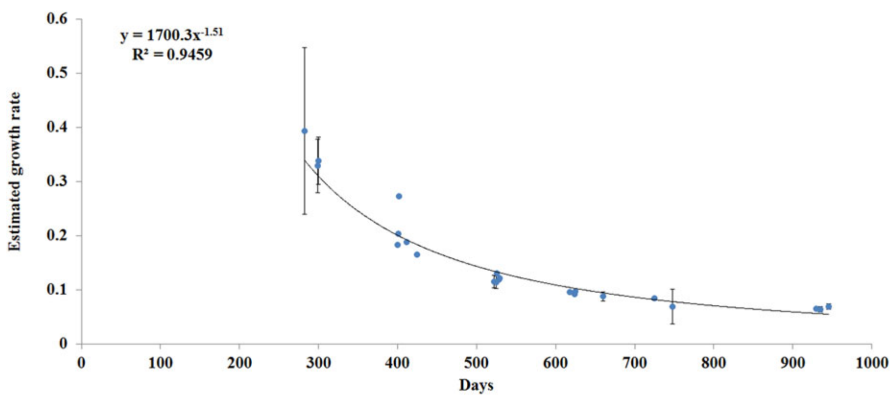

FIGURE 9. Estimated growth rate following hatch for Eastern hellbenders reared at SHFH from approximately 300 d to 1,000 d. The growth rate is based on the weights shown in Figure 6 and was calculated as the difference between the final and initial weights over time. Each data point represents multiple measurements recorded during a single sampling event on the same day that were then averaged; the error bars are SDs.

the animals. Given the length of time (2.5–3 years) that it takes hellbenders to reach the size at which they can be released, we recommend that captive culture areas be within a controlled environment without public access. Further, we have learned the carrying capacities of the specific culture space (Table 3). We have found that for the four RASs at our facility a minimum of one full-time employee is required for proper daily care. It is also

TABLE 3. Approximate number of Eastern hellbenders that can be held in a rearing tank (bottom area, 9,891 cm^2) at SHFH, by weight-, size-, and age-class. The parameters included in this table were established empirically at the Ron Goellner Center for Hellbender Conservation and the Wild-Care Institute at the Saint Louis Zoo. Abbreviations are as follows: SVL = snout-to-vent length, mo = months, and yr = years.

Weight class (g)	SVL (cm)	TL (cm)	Age	No. of hellbenders	Bottom area per hellbender (cm²)	Total weight (g)	Density (g/cm²)
≤10	≤6.8	≤11	6–8 mo	118	84	≤1,177.50	≤0.12
10–20	6.8–11.0	11.0–16.6	7 mo–2 yr	82	120	824.25–1,648.5	0.08–0.17
20–35	9.0–13.5	16.0–21.0	1.5–2.5 yr	59	167	1,184.55–2,072.96	0.12–0.21
35–60	12.5–16.0	20.0–25.0	2.5–4 yr	36	278	1,245.27–2,134.75	0.13–0.22
60–140	15.0–18.5	23.5–29.0	3–5 yr	24	418	1,419.76–3,312.78	0.14–0.33
140–250	15.5–21.5	24.0–36.0	4–7 yr	14	696	1,989.57–3,552.80	0.20–0.36
250–320	20.0–24.0	28.0–37.0	5–9 yr	9	1,044	2,368.53–3,031.72	0.24–0.31
320–500	20.0–25.0	28.0–38.0	7–9 yr	5	2,088	1,515.86–2,368.53	0.15–0.24
≥500	≥20.0	≥31.5	>9 yr	2	4,177	≥1,183.98	≥0.12

essential to develop beneficial support contacts with other entities involved with the captive culture of hellbenders. In addition to a strong biosecurity plan, the development of disease treatment protocols for bacteria, fungus, and water mold have assisted in providing appropriate diagnostic procedures and treatment regimens. For hellbender culture, we have previously observed the animals climbing vertical screens, so it is important to provide supplementary confinement by placing mesh screens on the drain pipes. The purpose of the Eastern hellbender propagation program in Missouri has been to augment existing populations, reduce the risk of extinction, and provide for the recovery and potential delisting of these animals from state-endangered status (Unger et al. 2013; Briggler et al. 2014). Our captive propagation techniques and protocols have improved over time, and the program is contributing an increasing number of animals to augment existing populations or provide specimens for research on the potential causes of population declines (Briggler et al. 2014). With an increasing need for the captive propagation of endangered species, the Missouri Department of Conservation is currently investigating the feasibility of constructing an Aquatic Species of Conservation Concern facility to increase the captive propagation space for these hellbenders and other species that are native to Missouri. The proposed facility would eliminate the need to retrofit an existing propagation facility for the specific needs of an endangered species, which is likely to reduce space for existing captive propagation efforts for sport fish.

ACKNOWLEDGMENTS

Funding for this study was provided by the Missouri Department of Conservation and the U.S. Forest Service. We thank the numerous Missouri Department of Conservation staff who assisted with the captive husbandry and collection of hellbender eggs from the wild. We also thank the University of Missouri Veterinary Medical Diagnostic Laboratory and the St. Louis Zoo herpetarium, veterinarian, and life support staff, who have provided assistance and support over the years. The mention of specific products in this article does not constitute endorsement by the Missouri Department of Conservation. There is no conflict of interest declared in this article.

ORCID

Timothy J. Bruce http://orcid.org/0000-0002-4690-6516

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