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One Hell of a Tale

A HOLISTIC MODEL FOR HELLBENDER CONSERVATION IN INDIANA

By Nicholas G. Burgmeier, Rod N. Williams and Sarabeth Klueh-Mundy

In midwestern and eastern states, an ancient amphibian quietly lives in the cool waters of rivers and streams hidden from view. The relatively unknown eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) has survived for nearly 2 million years, yet few people realize a remnant of the Pleistocene Epoch — a giant, fully aquatic salamander that lived at the same time as smilodons and mastodons — can still be found hiding beneath rocks.

But this unusual species has experienced a precipitous decline throughout its range over the past several decades (Wheeler et al. 2003; Burgmeier et al. 2011c). Biologists are uncertain of the cause, but the most likely contributing factors are increasingly poor water quality, stream sedimentation, impoundments caused by dams and persecution by uninformed recreationists (Gates et al. 1985; Mayasich et al. 2003). What is certain, however, is that in many areas throughout its range, the hellbender is on a clear path to extirpation unless proactive steps are taken (Unger et al. 2013b).

Previously found in many tributaries of the Ohio River in southern Indiana, the state's hellbender population now only persists in the Blue River — a 90-mile-long stream that runs through the southern counties of Harrison, Crawford and Washington — and possibly one of its tributaries, the South Fork Blue River. What's also troubling is that the Blue River population is geriatric, with no documented larvae or juveniles since the early 1980s (Kern 1984; IDNR, unpublished data).

To help save this species, the Indiana Department of Natural Resources (IDNR) received funds from the State and Tribal Wildlife Grants program of the U.S. Fish and Wildlife Service. In 2007, IDNR approached our research group at Purdue University about implementing a holistic recovery program that has now become a model for hellbender conservation nationwide. Our approach, which was based on three successive phases — population assessment, assistance and recovery — is now nearing the exciting point of reintroducing captive-reared salamanders into the wild.

Credit: Brian Tornabene

▼ **Purdue biologists conduct a snorkel survey to look for eastern hellbender males guarding their dens in the Blue River in southern Indiana. The researchers use dive lights to locate suitable nest rocks in hopes of finding a male hellbender poking its head out of the den.**

Phase I — The State of Things

In the summer of 2007, we started by investigating the current status of Indiana's hellbender population. The objectives were to estimate the existing population size, determine the home range and habitat use of residents, examine population health and habitat quality, and assess the genetic condition of the population.

After nearly 1,500 hours of sampling over the course of two years, the results were grim. We calculated a population density of only a fraction of an individual within a hundred square meters, which was at least an order of magnitude lower than other declining hellbender populations and far below estimates for more robust populations (Humphries and Pauley 2005). In addition, the population had a biased sex ratio of 2.6 males per female (Burgmeier et al. 2011c). Despite this intensive sampling effort covering Indiana's prime hellbender sites, we found only two nests and no subadults.

One of the major challenges facing the future of hellbenders in Indiana is the fact that the low

population density combined with a skewed sex ratio doesn't bode well when it comes to the species' reproduction prospects. On top of this, hellbenders are fairly sedentary. Our radio-telemetry studies showed that the salamanders are highly loyal to their home territory (Burgmeier et al. 2011a). In fact, we recaptured two individuals in 2015 within meters of their 2008 and 2009 capture locations. Even during the breeding season, hellbenders don't travel long distances and therefore few opportunities for reproducing exist.

However, some good news came from our initial assessment. The animals showed few signs of stress or disease and no reproductive anomalies (Burgmeier et al. 2011b).Water quality testing also showed the streams were healthy (Burgmeier et al. 2011b). Our DNA analyses of the salamanders revealed genetic diversity comparable to more robust populations and low levels of inbreeding (Unger et al. 2013a). With these results, we were encouraged to know that Indiana's population had some important components — adequate habitat, health and genetic stock — that would allow us to transition the project to the next step.

Phase II — Into the Wild

With baseline data collection completed, we had three immediate goals: develop a population viability analysis for the Blue River hellbender population, assess public perception and familiarity with hellbenders, and determine the viability of translocations.

The viability analysis told us that that a juvenile survival rate of 30 percent was needed to reduce the probability of extinction to near zero (Unger et al. 2013b). This rate is lower than the 50 percent reported in previous survivorship studies of translocated juveniles (Bodinof et al. 2012), so we felt confident we could increase the survival rate if we took the appropriate steps. More importantly, the analysis gave us a concrete target for population viability and growth.

Meanwhile, public outreach efforts also were underway. As a slimy and cryptic salamander, the hellbender does not typically garner the same positive reactions from the public as beautiful birds or cute mammals. In fact, anglers have long persecuted hellbenders because they believe the salamanders are poisonous and eat game fish. Given these negative public perceptions, we wanted to better understand stakeholders' awareness, attitudes and behaviors prior to developing our outreach materials.

Credit: Brian Tornabene

▲ **An eastern hellbender male guards the entrance to its den in Indiana's Blue River, the only river in the state with a population of the salamanders. Male hellbenders select suitable nest sites and guard them from competing males. Females enter the nest rocks and lay eggs, which the male then fertilizes. Males guard the eggs through hatching and into the early stages of larval development.**

We partnered with Purdue social scientists who sent a survey to landowners near the Blue River asking them about their familiarity with the hellbender. The results showed that this group was very familiar with the species: only around 5 percent of respondents reported negative behaviors like killing or throwing the amphibians on the river bank and collecting them (Mullendore et al. 2014). To build on these positive perceptions as well as to broaden the scope of our audience in Indiana, our group partnered with three Indiana zoos, Fort Wayne Children's Zoo, Mesker Park Zoo and Botanic Garden in Evansville, and Columbian Park Zoo in Lafayette to develop comprehensive outreach programs.

Based on our survey data, Purdue social scientists and our hellbender team created goals for educating the public about the current efforts to protect hellbenders and described their important role as an indicator species and how anyone can assist in protecting the species. This and other outreach efforts led to the creation of our website, HelptheHellbender.org, which now serves as the national hub for all hellbender-related research and outreach information.

To determine how hellbenders would cope with translocation, we moved 11 adult animals in 2011 from isolated areas within the Blue River to one of the river's best hellbender habitats. An additional 10 juveniles with similar genetic signatures (Unger et al. 2013a) — donated by the West Virginia

Department of Natural Resources — were placed upstream in a similar site.

Radio-telemetry data suggested that most individuals quickly took up residence in the area, with only three making long distance movements. In addition, about 76 percent of the translocated adults survived at least 732 days, which is similar to a previous estimate of 80 percent from another study of resident Blue River adults (Olson et al. 2013). The survival rate of translocated juveniles was lower — 22.5 percent lived 276 days — though a significant increase over our presumed pre-release survival rate of less than 1 percent.

Credit: Bart Kraus

▲ **A clutch of eastern hellbender eggs sits in an artificially created nest rock. Biologists designed the rock-like boxes with removable lids to allow them to easily check for and remove eggs without disturbing nesting habitat irreparably.**

With reproductive success critical to the hellbenders' survival, we needed a method to augment breeding habitat while also improving our survey efficiency. We have now placed 119 artificial nest boxes (Briggler and Ackerson 2012) in the river that simulate ideal breeding sites. The structures, which resemble rocks and have a lid, not only help increase nest search efficiency but also help reduce disturbances to the salamanders during surveys. Since placing the nesting structures in 2012, we have collected fertilized eggs from four boxes, which we then take to the lab to hatch and raise in captivity.

Phase III — Preparing for the Future

Based on our efforts, it was becoming clear that preserving Indiana's hellbender population would require novel approaches to boost the juvenile survival rate beyond the 30-percent threshold. Therefore, we initiated some additional projects focused on nest collection and captive-rearing, juvenile conditioning and reintroduction, microbiota analyses, river quality evaluations, and environmental DNA surveys.

From the summer of 2012 through the fall of 2015, our team snorkeled the Blue River searching for nests. In those four field seasons, we collected seven nests that have since yielded 345 healthy larval and juvenile hellbenders. As a part of the overall conservation strategy, we are now conditioning these captive-reared juveniles in the lab so that they are ready for the river environment and predators they will encounter upon release. Artificial streams allow the larvae to experience water currents and become acclimated to natural water flow conditions. We also plan to introduce individuals to natural predatory cues to elicit appropriate avoidance behavior and give them low doses of natural pathogens to improve their immune function. Our hope is that this rigorous conditioning will help increase the survival rate beyond the 30-percent benchmark needed to prevent extirpation.

In addition, we needed to understand more about the microbial conditions hellbenders are exposed to in the wild. Natural microbial conditions might differ from those of our lab-raised population and could include harmful infectious diseases. As a result, we are currently examining the microbiotic community that exists in the animals' bodies — as well as the microbiota of the Blue River — to determine what steps we could take to better prepare young hellbenders for reintroduction.

To ensure these larvae are placed in the best possible wild habitat, we are also evaluating habitat using ecological niche modeling. Juvenile and larval hellbenders require gravel and cobble substrates with clean spaces for shelter and foraging. To select translocation sites, we used side-scan sonar technology to make detailed maps of the river bottom for the Blue River. These data — combined with existing habitat and hellbender occurrence data — will be used to develop a niche model that predicts the best release sites. Once a release site has been chosen, we also plan to install supplementary cobble beds to help boost survival rates.

This summer, our current set of captive-reared, sub-adult hellbenders is scheduled to be released

into the clean cobble beds placed in the Blue River. To document survival rates, we plan to monitor this population by radio-telemetry.

With the entirety of Indiana's remaining hellbender population isolated to a single river, the population is very susceptible to catastrophic events and human collection (Nickerson and Briggler 2007). To avoid putting all our salamanders in one basket, so to speak, we also have begun to evaluate the suitability of several other historically occupied rivers for their repatriation potential.

It's important to note that reintroducing a species to unoccupied habitat is much more complicated than picking a location and releasing individuals. It is critical that every aspect of the habitat be evaluated to ensure the species has the best possible chance to thrive. After environmental DNA tests confirmed other rivers lacked hellbenders, we chose a candidate river for reintroduction. Before releasing any hellbenders in this river, evaluations of the water quality and crayfish populations will need to be completed to further assess the site.

The Future of Hellbender Conservation

With the first three phases of the study nearing completion, we are preparing for the next step in hellbender conservation. Our partnerships with zoos in Indiana have expanded, and they will now start raising a portion of our captive juvenile hellbenders. Also, after extensive consultations with hellbender researchers at Missouri's St. Louis Zoo who have had tremendous success with captive breeding of Ozark and eastern hellbenders, Indiana's Mesker Park Zoo and Botanic Garden is building its own artificial stream in an attempt to breed salamanders collected from the Blue River.

Last fall before the weather precluded further attempts, we successfully collected two adult females from isolated sites in the Blue River to use for the captive breeding program. Collections will resume this spring, hopefully in time for individuals to acclimate to their new habitat in the lab before the breeding season begins in the fall. If successful, a steady supply of subadult hellbenders raised from Indiana individuals will be released into the Blue River and possibly into other previously occupied rivers.

Through a multi-disciplinary collaboration of universities, zoos and government agencies and with funding support from the State and Tribal Wildlife Grants Program, we have been able to implement a truly holistic approach to the conservation of this highly specialized species. It is our hope that these efforts will provide a blueprint for other states to follow and help the eastern hellbender population stabilize and grow — not only in the Blue River but throughout the giant salamanders' historic range in eastern North America. ■

Credit: Brian Tornabene

▲ **Purdue University doctoral student Erin Kenison kneels next to the current homes of 235 juvenile hellbenders at Purdue University. These juveniles will eventually be released into Indiana's Blue River.**

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