STATE WILDLIFE GRANT—INDIANA
Assessing Hellbender Habitat for Potential Reintroductions

A male hellbender guards its nest rock. No eggs were found with this male. (Photo by Purdue University)

CURRENT STATUS
Third year of a four-year project

FUNDING SOURCES AND PARTNERS
State Wildlife Grant Program (T7R.18)
Purdue University

PROJECT PERSONNEL
Dr. Rod N. Williams, Principal Investigator,
Purdue University
Nick Burgmeier, Extension Wildlife Specialist,
Purdue University
Jenny Sutherland, Husbandry Technician, Purdue University
Brian Tornabene, Technician, Purdue University
Brianna Osinski, Technician, Purdue University
Helen Sung, Technician, Purdue University
Veronica Yager, Technician, Purdue University
Lauren Hendrickson, Technician, Purdue University

BACKGROUND AND OBJECTIVES
Populations of the eastern hellbender (Cryptobranchus alleganiensis) have declined dramatically throughout its range, with losses most severe in the Midwest. Populations in Ohio and Missouri continue to decline, and the species has been extirpated from Illinois and reduced to a single river in Indiana. Population abundance and density in Indiana are critically low, leaving hellbenders scattered throughout the river with little interaction among individuals. Consequently, there has been little, if any, confirmed reproduction for this population in the past two decades. Most recently, population viability analyses (PVA) indicate that, without efforts to increase juvenile recruitment and survival, the Indiana population is at risk of extirpation within 25 years.

Two recent studies in Indiana addressed restoration of hellbenders through head-starting and translocation
of juveniles (State Wildlife Grant T7R11: Repatriation of Eastern Hellbenders via Translocations and Headstarting; State Wildlife Grant T7R15: Assessing Juvenile Survival in Eastern Hellbenders). These efforts have increased survival in juvenile hellbenders to 30%, levels that PVA modeling suggest are adequate not only to prevent extirpation, but also to reverse long-term declines. These studies, however, were limited to the Blue River, where detailed occupancy and habitat information were available. Herein, we evaluate the potential to expand the Indiana distribution of hellbenders by assessing previously occupied habitats and using reintroductions.

More than 700 translocations, reintroductions and augmentations for various species are attempted annually in North America. The number is likely to increase as more species become threatened and endangered. More often, these techniques are being used to augment or reintroduce wild populations of amphibians and reptiles. Previous studies reported 31% of reintroduced amphibians can successfully breed in the wild, and 22% established and maintained self-sustaining populations. Despite these accomplishments, there may be questions or negative impacts that must be considered before reintroductions and augmentations are conducted. Those factors include, among others, the suitability of release sites (e.g., water quality, prey abundance, habitat availability) and the potential of introducing or spreading diseases and parasitic organisms.

There is a growing body of evidence of the negative impacts of chemical contaminants on amphibian populations. Chemical contaminants are easily absorbed through amphibian skin, and some species are far more susceptible than others. Hellbenders are particularly vulnerable because more than 90% of their respiration occurs through their highly vascularized skinfolds. However, the extent that hellbenders are affected by poor water quality remains unclear. A complete assessment of water quality and its biological impacts on hellbenders is needed before selecting hellbender reintroduction sites.

Declines in amphibian populations from disease
have received considerable attention in the past two decades. The organism(s) that cause disease are well known in some species but remains a mystery for others. As a result, it is important to describe the microorganisms that live on amphibians and in their environment to identify both harmful and beneficial bacteria and to record the overall microorganism community. Metagenomics is a method to describe the diversity of organisms in a community using environmental DNA (eDNA). The term eDNA refers to the practice of testing samples collected from the environment, such as soil or water, for the DNA of a specific organism. This approach allows researchers to passively sample for species presence in an area without the need to encounter the animal. Depending on the chosen methods, this approach can describe the nearly entire biodiversity of the environment in a completely non-invasive manner.

Because disease is a major factor causing decline in amphibians, a thorough understanding of the microbial community between source and supplemented populations can reduce the risk of transferring pathogens and provide information regarding the quality of the habitat when introducing individuals. Identification of all community organisms using eDNA provides information on potential parasites, predators, competitors, and prey that might interact with hellbenders. Future reintroductions of captive-reared individuals in Indiana must assess water quality, prey abundance, habitat availability, and the plant and animal species present in source habitat, reintroduction sites, and rearing facilities to fully evaluate post-release survival. These factors underscore the importance of studying the biology and ecology of the candidate species before and immediately after any reintroduction.

It is important to evaluate historical habitats to better understand the distribution of hellbenders in Indiana and the potential for the species to survive in new habitats. We will use eDNA in historically occupied streams (Fourteenmile Creek, Little Blue River, Laughery Creek, South Fork of the Blue River, Indian Creek) to determine if hellbenders still occur in these systems. We will also evaluate these streams for habitat suitability (i.e., water quality, prey abundance, microbiota) and identify specific sites within suitable streams for reintroduction. Upon reintroduction, all individuals will be monitored to determine home range size, habitat use, and post-release survival. The objectives of this project are to:

*Purdue research technician Lauren Hendrickson collects a water sample from Indian Creek for pesticide analysis.* (Photo by Purdue University)
1. Sample historic and select rivers for the presence of hellbenders using eDNA.
2. Assess habitat suitability in the Little Blue River, Indian Creek, Fourteenmile Creek, Laughery Creek, and South Fork of the Blue River.
3. Identify suitable sites for reintroductions of captive-reared juveniles.
4. Assess home range size, habitat use, and post-release survival of reintroduced hellbenders.

**METHODS**

Water samples from the Little Blue River, Fourteenmile Creek, South Fork of the Blue River, Laughery Creek, and Indian Creek were tested for presence/absence of hellbenders using eDNA methods. Streams were sampled at three sites equally distributed along the length of each stream; five sites were sampled on Indian Creek given the greater length of this stream. At each site, we collected two liters of water and tested each in triplicate (six tests/site) for the presence of hellbender eDNA.

To determine which, if any, rivers are suitable for hellbender reintroduction, we examined physical habitat characteristics and organismal communities using molecular approaches. We floated the length of each river (126 total river miles) to assess the presence of hellbender habitat. We classified and ranked sections of each river by the flow type (e.g., pool, riffle, run), amount of large boulders, and substrate type (e.g., gravel, cobble, bedrock, silt, sand). We used mapping software to compile the rankings and highlight which areas had the highest quality habitat. We then used eDNA to compare biological communities between sample locations. We collected two eDNA samples each from the Little Blue River, South Fork of Blue River, Laughery Creek, Indian Creek, and Blue River. We sequenced all samples in triplicate to characterize the entire biological community at each site and used metrics to compare community structure across potential reintroduction rivers. Finally, crayfish surveys were conducted to evaluate prey base.

Suitable sites in Indian Creek were evaluated for water quality and prey items. Water quality samples were collected from three sites on four separate sampling occasions in Indian Creek. Samples were collected from the water column and fine substrate deposits to determine if contaminants were present and to ensure suitable physical properties. Macroinvertebrates and crayfish were sampled following Indiana Department of Environmental Management protocols to ensure suitable prey abundance. Sampling was not conducted in the other four rivers because Indian Creek was chosen as the top candidate for future release.

After all habitat evaluations have been made, and if a suitable site has been found, 40 juveniles will be released. All juveniles will have implanted radio-transmitters to allow post-release monitoring for six to nine months. We will use radio-telemetry to determine home range sizes, habitat use and survival to help evaluate whether captive-rearing techniques were successful.

**PROGRESS TO DATE**

In summer 2015, we evaluated five historically occupied streams for hellbender habitat. The Little Blue River and Fourteenmile Creek lacked sufficient suitable habitat for reintroduction. The South Fork of Blue River had excellent habitat but excessive siltation would likely prevent establishment of viable populations. Laughery Creek also had good habitat, but it is a shallow and warmer stream with substantial recreational activity, including off-road vehicles. Indian Creek was comparable to the Blue River in habitat quality but also has areas with high algal growth and several dams and areas with subsurface flow. The dams are being considered for removal in 2019 or 2020, at which point Indian Creek will be further explored as a reintroduction option.

We collected 14,522 macroinvertebrates from four sites in Indian Creek and four sites in the Blue River in summer 2016. The resulting comparison of macroinvertebrates indicates that Indian Creek and Blue River share similar high quality habitat and water quality traits. Crayfish densities were comparable (Blue River: 3.19/m²; Indian Creek: 3.31/m²), which suggests that Indian Creek provides an adequate food source for hellbenders. Further, eDNA results indicate that the biological community in Indian Creek is nearly indistinguishable from that in the Blue River. Sequencing results detected 1,136 eukaryotic species across 19 phyla throughout the sample area. Of potential reintroduction rivers, Indian Creek had the most comparable biological community to the Blue River, followed by the South Fork of the Blue River and Fourteenmile Creek. The Little Blue River and Laughery Creek both differed greatly in community structure compared to the three other rivers.

Seventeen eDNA samples from the five historic streams were collected in fall 2015, none of which tested positive for hellbender DNA. It is unlikely that hellbenders persist in these streams, although it is possible they remain in numbers undetectable using eDNA.

Water quality and sediment sampling was completed in Indian Creek in 2018. All physical properties of the water (i.e., dissolved oxygen, specific conductivity, pH, and temperature) were generally within suitable levels to support a healthy biotic community. However, there was one concerning decrease in dissolved oxygen (4.71 mg/L) at one site in August that warrants future monitoring. No organochlorine or organophosphorus pesticides were detected in the water column. No heavy metals were detected in the water, but several were detected in sediment samples from all sites. Cadmium and mercury were not detected in water or sediment samples. Low levels of nitrogen were present...
in all water and sediment samples at all sites. Low levels of total phosphorus were detected in some water samples and one sediment sample during the summer months. No polychlorinated biphenyls (PCBs) were detected in sediment samples, but polycyclic aromatic hydrocarbons (PAHs) were detected in all sediment samples. Only two PAHs were detected in the sediment sample taken upstream from the town of Corydon. However, nine PAHs were detected in the sampling site immediately downstream from Corydon, and six were detected in the site farther downstream.

Searches for egg masses in the Blue River continued in 2018. However, due to high water and poor water clarity throughout the nesting season, fewer surveys were conducted than in years past. Four sites were surveyed three times each, one was surveyed twice and an additional two sites were surveyed once each. Two guarding males were found at two separate sites. One guarding male was found in an artificial nest box but no eggs were found. An additional guarding male was found under a natural bedrock ledge but high water flows prevented probing attempts. However, an adult female hellbender was collected and transported to Mesker Park Zoo and Botanic Garden for inclusion in a captive breeding program.

We are preparing for the 2019 season, which will include data analysis on water quality samples from Indian Creek. We will continue to explore this creek as a potential reintroduction stream and examine each potential release site at varying water levels. The potential removal of dams on Indian Creek will prevent any releases in 2019 and will likely delay release into Indian Creek until 2021 (at the earliest). Dam removals can produce unexpected changes in river condition and could cause decreased survival in released individuals; however, the removal of the dams would likely yield a more suitable system for hellbenders in the long term.

COST: $654,213 FOR THE COMPLETE FOUR-YEAR PROJECT