STATE WILDLIFE GRANT—INDIANA
Assessing Hellbender Habitat for Potential Reintroductions

Purdue biologists conduct crayfish abundance surveys in Indian Creek to evaluate if the prey base is suitable for hellbender reintroduction. (Photo by Erin Yager)

CURRENT STATUS

Second year of a four-year project

FUNDING SOURCES AND PARTNERS

State Wildlife Grant Program (T7R18)
Purdue University

PROJECT PERSONNEL

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BACKGROUND AND OBJECTIVES

Populations of the Eastern hellbender have declined dramatically throughout the species' range with losses most severe in the Midwest. Populations in Ohio and Missouri continue to decline. The species has been extirpated from Illinois and is now only found in the Blue 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 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 Head-starting; State Wildlife Grant T7R15: Assessing Juvenile Survival in Eastern Hellbenders). These efforts have

increased survival in juvenile hellbenders by 30%. PVA modeling suggests these levels are adequate to not only 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 doing 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. Most 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—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 hellbender reintroduction sites are selected.

Declines in amphibian populations from disease have received considerable attention in the past two decades. The organism(s) that cause disease are well known for 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 used by biologists to describe the diversity of organisms in a community using environmental DNA (eDNA). eDNA is 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 needing to encounter the animal. Depending on the chosen methods, this approach can describe nearly the 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 reintroduction candidate species before and immediately after 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.

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 because of its greater length. At each site, we collected two liters of river 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

This large dam on Indian Creek that is being considered for removal to improve river connectivity and hellbender habitat. (Photo by Nick Burgmeier)

community structure across potential reintroduction rivers. Finally, crayfish surveys were conducted to evaluate prey base.

Suitable sites will be further evaluated for water quality and prey items. Samples will be taken from the water column and substrate to determine if contaminants are present and to ensure suitable physical properties. Macroinvertebrates and crayfish will be sampled after Indiana Department of Environmental Management (IDEM) protocols to ensure suitable prey abundance.

After all habitat evaluations have been made and a final site selected, 40 juveniles will be released. All juveniles will have implanted radio-transmitters to allow six to nine months of post-release monitoring. We will use radio-telemetry to determine home range size, habitat use, and survival to evaluate the success of our captive-rearing technique.

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 its excessive siltation would likely preclude establishment of viable populations. Laughery Creek also had good habitat, but it is a shallow and warmer stream with substantial recreational activity, including use of 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, and Indian Creek will be further explored as a reintroduction option.

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 they may remain in numbers that are undetectable using eDNA.

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 Indian Creek and the Blue River share similar high quality habitat and water quality traits. Crayfish densities were comparable (Blue River: 3.19/m^2; Indian Creek: 3.31/m^2) which suggests that Indian Creek provides an adequate food source for hellbender reintroduction. Furthermore, 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's, 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 that of the three other rivers.

Searches for egg masses in the Blue River continued in 2017. Eight sites were surveyed four times each for a total of 42 hours. Three guarding males were found at three separate sites. Each potential nest rock with a guarding male was probed up to three times to search for eggs, but no nests were found. We will continue to monitor these sites throughout fall to account for possible late breeding events.

We are preparing for the 2018 season, which will include the continued classification of macroinvertebrates and the collection of 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 likely delay any releases in 2018 and could possibly eliminate it as a reintroduction stream in the near future. 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 hellbender repatriation in the long term.

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