Developing a coordinated research approach for hellbender conservation in the northeast with benefits to wild mudpuppy populations

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RCN funds requested: $99,886 **Project duration: January 2014 – December 2015**

Regional Conservation Needs Topic 3: Identify NE Species of Greatest Conservation Need Data Gaps, Design Data Collection Protocols, and Collect Data

Project Summary:
Although the hellbender has been identified as a *Species of Greatest Conservation Need* by the Northeast Association of Fish and Wildlife Agencies, there remain substantial data gaps in its distribution. The common mudpuppy shares a significant portion of its habitat with the hellbender, and has been identified as a *Species of High Conservation Concern* by the Northeast Partners in Amphibian and Reptile Conservation. Given the habitat overlap of these two species, efforts to detect hellbenders may concurrently generate samples or data that are useful in monitoring mudpuppy populations. Our primary objectives are to 1) **better document hellbender distribution** in the northeast region, and 2) **develop standardized methodologies** to monitor hellbender populations while collecting opportunistic information about mudpuppy distribution. These objectives will be achieved through stream surveys (including environmental DNA detection), improved communication among individuals working with hellbenders or mudpuppies, and the establishment of a regional stakeholder working group. Within the first year of the project we will produce standardized protocols that ensure the consistency and efficiency of hellbender/mudpuppy surveys while minimizing disturbance of stream boulder habitat. During this time we will also collect environmental DNA (eDNA) samples from a total of ~130 sites in NY, PA, MD, WV, and VA. Samples will be tested for hellbender DNA and archived for future DNA-based detection of mudpuppies or other stream species. In the second year, we will employ conventional surveys to 'ground-truth' a subset of eDNA sites. This approach will generate presence/absence data for a broad geographical area and information about abundance, demographics and animal health for a key subset of sites. Project deliverables include 1) a more comprehensive map of hellbender distribution in the northeast, 2) an eDNA archive (for detection of other stream-dwelling species) and 3) a protocol and communication framework to enable coordinated and efficient conservation of hellbenders and mudpuppies.

Background

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is declining in many parts of its range [1] and has been identified as a *Species of Greatest Conservation Need* by the Northeast Association of Fish and Wildlife Agencies (NAFWA). The species' historic range in the northeast includes New York, Pennsylvania, Maryland, West Virginia and Virginia. Despite a significant amount of research effort, substantial gaps remain in our knowledge of the hellbender's current distribution, particularly in NY, PA and VA. Given the broad distribution and cryptic nature of this species, generating a comprehensive distribution map will be challenging (and likely not feasible in the near future) using traditional approaches. Yet such information is urgently needed to guide ongoing efforts to protect and restore wild hellbender populations. Environmental DNA (eDNA) analysis is a relatively new approach to determining the presence/absence of aquatic vertebrates in targeted locations [2]. Since 2008, eDNA has been used successfully to establish distributions of freshwater species [3] and to detect invasive fish [4] and amphibians [5], including stream-dwelling species [6]. In Georgia and Tennessee, eDNA analysis identified previously unknown hellbender populations that were later confirmed by conventional stream surveys (S. Spear, *personal comm*.). Although it is not yet clear if this approach can provide accurate estimates of abundance, at a minimum eDNA would provide reliable presence/absence data to greatly facilitate efforts to address distribution data gaps.

Hellbender conservation efforts in the northeast would also benefit from better coordination among researchers and population managers. Certain states (most notably WV) have well-established hellbender restoration programs, and this knowledge and experience will be valuable to others (e.g., VA) that are beginning to plan similar efforts. Furthermore, data or biological samples often can be collected more efficiently through state partnerships. For example, researchers in VA and WV are collecting blood from hellbenders for hormone analysis, and these samples could provide a source of DNA at no cost to Dr. McMillan, who is investigating the genetics of the northeastern population. Similarly, researchers in the northeast often encounter common mudpuppies (*Necturus maculosus*) during hellbender population surveys. Yet these opportunistic encounters generally are not recorded, representing a loss of systematic survey data for the species. This lost information is important because the common mudpuppy has declined in many areas and is considered a *Species of High Conservation Concern* by the Northeast Partners in Amphibian and Reptile Conservation [7]

The overall goal of this project is to increase knowledge of hellbender distribution in the northeast using a coordinated approach that captures opportunistic information about common mudpuppy populations. Our specific objectives are to 1) fill major data gaps in hellbender distribution throughout the northeast, and 2) develop efficient, standardized protocols for hellbender research and monitoring. To address the first objective, we will employ a two-tiered strategy to hellbender surveys that incorporates both DNA-based detection and conventional methods (i.e., snorkeling and rock-turning). This approach will provide hellbender presence/absence data across a broad geographic area and more detailed demographic information for a subset of these populations. Importantly, after screening for hellbenders, eDNA samples will be catalogued and archived for future detection of mudpuppies or other aquatic species. To address the second objective, we will develop a communication framework (including face-to-face meetings) for hellbender researchers and population managers working in the northeast. This framework will allow us to develop and disseminate protocols that

maximize the success of hellbender conservation efforts, while ensuring opportunistic benefits to other species (e.g., mudpuppy). Through these collective efforts, the project will address Topic 3 of the NAFWA's Regional Conservation Needs framework: *Identify NE Species of Greatest Conservation Need Data Gaps, Design Data Collection Protocols, and Collect Data*.

Methods and Timeline

Protocol development (Jan 2014 – March 2015)

Project partners will convene a special session at the June 2013 Hellbender Symposium (Chattanooga, TN) to outline the basic structure of the monitoring protocols and to identify a leader for each of the following topic areas: 1) conventional population surveys and occupancy modeling, 2) eDNA surveys, 3) disease/biomaterial sampling, 4) captive husbandry/propagation and 5) reintroduction and site restoration. Our top priority will be to establish a standardized eDNA protocol that will be used to collect samples in year 1 of the project. Project partners will meet twice during the project period (Jan 2014 and 2015) to further develop and subsequently evaluate research and monitoring protocols. Prior to the project's completion, finalized protocols will be distributed broadly via existing NGO channels (e.g., the Cryptobranchid Interest group listserve) as well as through state and federal wildlife agencies. A webpage will be established to serve as a centralized repository for the finalized protocols.

Environmental DNA (eDNA) surveys (April – Sep 2014)

All field work will be planned, coordinated and conducted in an ethical manner to avoid/minimize disturbance to animals and prevent disease spread between visited sites. Permits will be obtained prior to initiation of the study and a detailed protocol outlining all methods of the study will be approved by the workgroup based on Institutional Animal Care and Use Committee (IACUC) guidelines. Stream water (2 L) will be collected from a total of 130 sites across the northeast, broken down by state based on the relative extent of distribution data gaps: NY = 30, VA = 30, PA = 45, WV = 15, and MD = 10. Each partner will determine the optimal configuration of sampling sites, given the geographical extent of data gaps in his/her state and sampling feasibility/travel costs. Water samples will be collected from each site in triplicate, vacuum filtered, and stored at -20°C until analysis. The resulting filter papers will be analyzed for hellbender eDNA by quantitative PCR (qPCR) following established methods [8]. Dr. McMillan will be responsible for analyzing samples from NY and PA, and Dr. Greathouse will analyze those from VA, WV and MD. Detailed protocols for eDNA sampling and analysis will be developed at the Jan 2014 partner meeting.

Conventional hellbender surveys (May – Aug 2015)

A subset of sites testing positive for hellbender eDNA will be selected for 'ground-truthing' surveys using a conventional approach (i.e., snorkeling and rock turning). A detailed survey protocol will be developed at the Jan 2014 meeting of project partners and finalized at the Jan 2015 meeting. If hellbenders are not detected during a survey, the site will be resurveyed up to two times. The final number of sites surveyed per state will depend on the number of repeat surveys needed, but we will aim for the following breakdown of sites: NY = 6, VA = 6, PA = 9, WV = 3, MD = 1. Additionally, we will survey 1-2 sites per state where hellbender eDNA was not detected to serve as negative controls.

Project outcomes and deliverables

Locating hellbender populations in the northeast is the first step to protecting these species in this region and ultimately preventing their listing under the U.S. Endangered Species Act. Given the time and resources needed for extensive hellbender surveys, eDNA analysis and the coordination of ongoing research efforts are the most efficient and immediate actions that can be taken to help prevent its listing. Although we are investigating the potential to generate coarse abundance estimates through eDNA analysis, we are taking a conservative approach by relying on this technology for simple presence/absence data. At a minimum, eDNA data can be used to prioritize streams containing hellbenders for more in-depth, resource-intensive surveys.

The specific deliverables from this project include:

1. A more comprehensive map of hellbender distribution in the northeast
2. An archive of eDNA samples that could be used to establish distributions for other freshwater species of conservation concern (e.g., mudpuppy, wood turtle or bog turtle)
3. An effective communication framework for hellbender researchers and population managers working in the northeast
4. Standardized protocols for hellbender conservation efforts
    a. Conventional population surveys (including guidelines for minimizing habitat disturbance and capturing opportunistic data)
    b. Statistical methods for estimating population size and stability
    c. Unconventional survey methods (including eDNA)
    d. Biomaterial/disease sampling
    e. Captive husbandry and propagation
    f. Reintroduction and site restoration

References

1. Mayasich, J., D. Grandmaison, and C. Phillips, *Eastern hellbender status assessment report*. 2003, Duluth, MN: Natural Resources Research Institute.
2. Ficetola, G.F., et al., *Species detection using environmental DNA from water samples.* Biology Letters, 2008. **4**(4): p. 423-425.
3. Lodge, D.M., et al., *Conservation in a cup of water: estimating biodiversity and population abundance from environmental DNA.* Molecular Ecology, 2012. **21**(11): p. 2555-2558.
4. Jerde, C.L., et al., *"Sight-unseen" detection of rare aquatic species using environmental DNA.* Conservation Letters, 2011. **4**(2): p. 150-157.
5. Dejean, T., et al., *Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog Lithobates catesbeianus.* Journal of Applied Ecology, 2012. **49**(4): p. 953-959.
6. Goldberg, C.S., et al., *Molecular Detection of Vertebrates in Stream Water: A Demonstration Using Rocky Mountain Tailed Frogs and Idaho Giant Salamanders.* PLoS ONE, 2011. **6**(7): p. 5.
7. NEPARC, *Northeast Amphibian and Reptile Species of Regional Responsibility and Conservation Concern*, 2010.
8. Thomsen, P.F., et al., *Monitoring endangered freshwater biodiversity using environmental DNA.* Molecular Ecology, 2012. **21**(11): p. 2565-2573.

Project Budget	Year 1	Year 2
Direct costs		
Dr. Terrell (project leader) salary (based on 15% time commitment)	$7,500	$7,500
Fringe benefits for Dr. Terrell (30%)	$2,250	$2,250
Meeting travel (4 individuals representing VA and MD)	$1,600	$1,600
Travel and lodging for VA and MD eDNA sampling (35 sites)	$3,300	
Travel and lodging for VA and MD stream surveys (10 sites)		$6,668
Subcontract to Buffalo State College ($19,773 total)		
Meeting travel	$800	$800
Student salary for eDNA sampling and hellbender surveys	$2,500	$2,000
5% fringe benefits	$125	$100
Travel and lodging for eDNA (Yr 1) and hellbender (Yr 2) surveys	$1,595	$1,595
eDNA analysis (NY and PA sites, 225 samples total)	$7,679	
Indirect costs (15% of direct costs)	$1,905	$674
Subcontract to Western Pennsylvania Conservancy ($15,800 total)		
Meeting travel (2 individuals representing PA)	$800	$800
Travel and materials for PA eDNA sampling (45 sites)	$3,200	
PA hellbender surveys (11 sites)		$11,000
Subcontract to The Wilds ($15,146 total)		
Meeting travel (1 individual representing WV)	$400	$400
Travel and materials for WV eDNA sampling (15 sites)	$1,800	
Graduate student stipend for eDNA sampling	$3,500	
eDNA analysis (WV, MD, and VA sites, 165 samples total)	$5,346	
WV hellbender surveys (4 sites)		$3,700
Total direct costs	**$44,300**	**$39,087**
Indirect costs		
Smithsonian grants & contracts fee (28.3% of personnel costs)	$2,759	$2,759
Smithsonian general overhead (8.6% of direct costs and G&C fee)	$4,047	$3,559
Smithsonian Institutional management/support fee (4% of direct costs)	$1,772	$1,563
Total by year	$52,878	$47,008
GRAND TOTAL	**$99,886**	

Source of non-federal match	Amount
Buffalo State College	
A. McMillan salary (5%)	$7,264
50.16% fringe benefits	$3,644
Indirect costs (47% on match)	$5,126
Unrecovered indirect costs (32%)	$5,506
Virginia DGIF	
J. Kleopfer travel for surveys	$2,000
The Wilds	
Student salary for eDNA analysis	$5,761
J. Greathouse salary (4%)	$2,650
Program assistant salary (8%)	$1,768
Equipment and lab usage (eDNA)	$4,967
Western PA Conservancy	
Staff salary	$15,800
Smithsonian's National Zoo	
eDNA supplies*	$3,415
Hellbender survey supplies*,1	$10,305
2 unpaid, part-time interns**	$31,680
Total matching costs	**$99,886**

*Through a grant from the Society for Conservation Biology

[1]Includes wet suits, snorkels, nets, peavies, dry-shipper unit, water quality meter.

**Intern time: $8.25/hr * 40 hours/week * 24 weeks/year *2years

Dr. Kimberly Terrell is a wildlife physiologist at Smithsonian's Conservation Biology Institute (Washington, DC). She earned a Ph.D. in Conservation Biology from the University of New Orleans, and has nearly a decade of experience in physiological research. Dr. Terrell also has conducted physiological assessments of hellbender populations throughout the species' range, establishing an extensive network of research partners. At the Smithsonian's National Zoo, she is leading a captive-based research program to identify the impacts of climate change on hellbender physiology. She will be responsible for coordinating the project, as well as protocol development and sampling/survey work in Virginia.

John D. Kleopfer is a herpetologist with the Virginia Department of Game and Inland Fisheries. He earned a M.S. in Environmental Science from Christopher Newport University. For the past 8 years, he has directly supervised and/or managed numerous herpetological conservation and research efforts, including *A Multiple-Scale Assessment of Eastern Hellbender Populations in Virginia, including occupancy, abundance, habitat selection and physiological condition* with Dr. Bill Hopkins of Virginia Tech. Mr. Kleopfer will be responsible for developing and disseminating hellbender research and monitoring protocols.

Dr. Amy McMillan is an Associate Professor of Biology at Buffalo State College (Buffalo, NY). She earned a Ph.D. in Ecology and Entomology from the University of Kansas. Her research interests include population and conservation genetics. She has been working with hellbenders since 2003 and currently advises four graduate students studying distribution, genetics and/or reintroduction of this species. Dr. McMillan will be responsible for survey work in New York and eDNA analyses (NY and PA), and will contribute to protocol development.

Eric Chapman is the Director of Aquatic Science for the Western Pennsylvania Conservancy (Indiana, PA). He earned a B.S. in Biology from Indiana University of Pennsylvania, and a M.A. from Montclair State University in Environmental Studies. His research interests include freshwater mussel population dynamics, fish community structure in small streams, and hellbender population monitoring. His hellbender research focuses on mark-recapture surveys, growth trends of adult animals, and Chytrid fungus testing with Clarion University, in the Allegheny drainage of Pennsylvania. He will be responsible for survey work in Pennsylvania and will contribute to protocol development.

Dr. Joe Greathouse is the Director of Wildlife and Conservation Medicine at The Wilds (Cumberland, OH) and leads hellbender conservation efforts in West Virginia. He earned a B.A. in Biology, an M.S. in Wildlife and Fisheries Resources Management, and a Ph.D. in Animal and Food Sciences from West Virginia University. He is the Eastern Hellbender Taxon Champion for the Association of Zoos and Aquariums' Amphibian Taxon Advisory Group. He has been studying hellbenders in West Virginia since 2004 and, in 2007, successfully hatched the first hellbenders in captivity as the curator at Oglebay Zoo (Wheeling, WV). Dr. Greathouse will be responsible for analysis of eDNA samples from VA, WV and MD, and for WV sampling/survey work. He will also contribute to protocol development.

Edward Thompson is a wildlife biologist with the Maryland Department of Natural Resources. He has been monitoring the state's hellbender populations for the past 20 years. He will contribute to protocol development and will coordinate sampling/surveying in MD.