The 7th Biennial Hellbender Symposium

June 14 – 17, 2015

Saint Louis, Missouri

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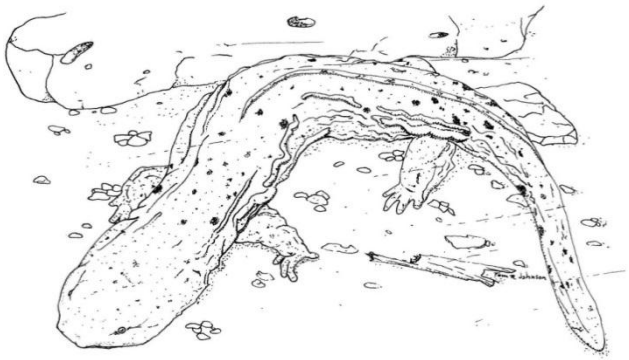

7th Hellbender Symposium Program

June 14 – June 17, 2015

Sunday, June 14 (Sheraton Clayton Plaza Hotel)

1:00 – 6:00 p.m.	Registration open at the Sheraton Clayton Plaza Hotel
6:00 – 7:00 p.m.	Icebreaker (Grand Ballroom)
7:00 – 7:15 p.m.	Welcoming remarks (Grand Ballroom)
7:15 – 8:00 p.m.	Dinner (Grand Ballroom)
8:00 – 9:00 p.m.	Keynote Address (Grand Ballroom) - **Separating the Wheat from the Chaff: Evaluating the Knowing and Doing of Hellbender Recovery** Gregory Lipps, Program Manager, Amphibian & Reptile Conservation, The Ohio State University
9:00 – 9:30 p.m.	Q&A – Hands-on workshop overview and group assignments for Monday, June 15

Monday, June 15 (Saint Louis Zoo)

7:00 – 8:30 a.m.	Registration open at the Sheraton Clayton Plaza Hotel
8:30 a.m.	Buses begin to depart for the Saint Louis Zoo for the Hands-on Workshop
8:00 – 6:30 p.m.	T-shirts available for sale at the Group Ticket Desk (The Living World)
9:00 – 9:30 a.m.	Continental breakfast at the Saint Louis Zoo (Event Tent)
9:30 – 12:30 p.m.	Hands-on workshop (Event Tent & Charles H. Hoessle Herpetarium)
12:30 – 1:30 p.m.	Lunch (Event Tent)
1:30 – 3:30 p.m.	Hands-on workshop (Event Tent & Charles H. Hoessle Herpetarium)
4:00 p.m.	Workshop wrap-up (Event Tent)
5:00 p.m.	Group photo (Sea Lion Sound)
5:30 – 6:15 p.m.	Reception (The Living World)
6:15 – 7:00 p.m.	Dinner (The Living World)
7:00 – 9:00 p.m.	State and Federal updates
9:00 p.m.	Buses depart for the Sheraton Clayton Plaza Hotel

Tuesday, June 16 (Sheraton Clayton Plaza Hotel)

7:30 – 8:30 a.m.	Continental Breakfast (Gallery Ballroom)
7:30 – 8:30 a.m.	Poster Presentation Session Setup (Grand Ballroom)
8:30 – 5:00 p.m.	Oral Presentation Session (Gallery Ballroom)

Moderator: Jeff Briggler

8:30 – 8:45 **Hellbender Monitoring and Recovery Efforts in Missouri**
Jeffrey T. Briggler

8:45 – 9:00 **Aspects of Eastern Hellbender Natural History from North-Central Pennsylvania**
Peter J. Petokas

9:00 – 9:15 **Nine Years of Eastern Hellbender Research in the Susquehanna River Watershed: Status of Extant Populations**
Peter J. Petokas

9:15 – 9:30 **Eighteen years of Natural History Observations of Hellbenders in the Appalachian Mountains: Nesting Habitat, Breeding Behavior, Growth, and the Conservation Costs of Lifting Rocks**
Jeff Humphries

9:30 – 9:45 **Searching for a Needle in a Haystack: Assessing the Distribution of Hellbenders in New York's Susquehanna River Drainage**
Robin Foster, Amy McMillan, and Chris Larsen

9:45 – 10:00 **Physiography is a Stronger Predictor of Hellbender Occupancy in Virginia than Land Use**
Catherine M. Bodinof Jachowski, Joshua J. Millspaugh, and William A. Hopkins

10:00 – 10:30 Break (Gallery Ballroom)

Moderator: Rod Williams

10:30 – 10:45 **Forest Removal: A Major Factor in the Decline of Hellbender Populations**
Max A. Nickerson, Amber L. Pitt, and Joseph J. Tavano

10:45 – 11:00 **Nest-guarding Behavior of a Male Ozark Hellbender in a Natural Habitat**
Rachel Settle, Jeff Briggler, and Alicia Mathis

11:00 – 11:15 **The Breeding Migration and Home Range Size of the Japanese Giant Salamander, *Andrias japonicas*, in a Headwater Stream with Special Reference to Den-masters**
Sumio Okada, Tamami Okada, and Zach Felix

11:15 – 11:30 **Growth of a Long-lived Salamander in the Field: Over the 40 Years Study of More than 900 Identified Japanese Giant Salamanders, *Andrias japonicus* in Hyogo Prefecture**
Yuki Taguchi and Takeyoshi Tochimoto

11:30 – 11:45 **The Use of Nest Boxes by the Hellbender Salamander in Western North Carolina**
Arianne Messerman, J.J. Apodaca, Lori Williams, Mark Endries, and Benjamin Prater

11:45 – 12:00 **Hydrodynamic Redesign of Hellbender Salamander Nest Boxes**
Mohammed G. Mohammed, Kathleen M. Trauth, and Arianne F. Messerman

12:00-1:15 p.m. Lunch (Grand Ballroom)

Moderator: Trisha Crabill

1:15 – 1:30 **Propagation Efforts for the Endangered Hellbender at the Saint Louis Zoo**
Jeffery A. Ettling and Jeffrey T. Briggler

1:30 – 1:45 **Egg Incubation and Early Stage Larval Rearing of Hellbenders**
Chawna Schuette

1:45 – 2:00 **Response to a Limited Feeding Regime in the Japanese Giant Salamander, *Andrias japonicus* at the Conservation Breeding Center, Asa Zoo, Hiroshima**
Yuki Taguchi and Noriyuki Nonoue

2:00 – 2:15 **Captive-release Hellbender (*Cryptobranchus alleganiensis alleganiensis*) Movement in Two Northern Allegheny Streams**
Julie A. Boerner, Amy M. McMillan, Robert J. Warren, II, and Kenneth J. Roblee

2:15 – 2:30 **Survival, Movement, and Shelter Selection of Reintroduced and Translocated Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in West Virginia Following Rearing in Human Care**
Joe Greathouse and Eugene Felton

2:30 – 2:45 **Post-release survival of 31 Reintroduced Captive-reared Chinese Giant salamanders (*Andrias davidianus*)**
L. Zhang, W. Jiang, H. Zhang, Q. Wang, H. Zhao, Ruth Marcec, S. Willard, and A. Kouba

2:45 – 3:00 **How Population Genetics and Parentage Analysis Can Inform Headstarting Efforts**
Amy McMillan, Meghan Jensen, and Robin Foster

3:00-3:30 p.m. Break (Gallery Ballroom)

Moderator: Amy McMillan

3:30 – 3:45 **Update on Environmental DNA Monitoring for Hellbenders in Georgia, North Carolina, and Tennessee**
Stephen Spear, Michael Freake, Lori Williams, John Groves, Thomas Floyd, Christopher Jenkins, and Lisette Waits

3:45 – 4:00 **Discovery and Utility of Sex-linked Genes in Cryptobranchid Salamanders**
Paul M. Hime, Schyler O. Nunziata, Jeffrey T. Briggler, Joshua S. Reece, Alan R. Lemmon, and David W. Weisrock

4:00 – 4:15 **Genome-scale Resolution of Species Boundaries and Demography in *Cryptobranchus***
Paul M. Hime, Jeffrey T. Briggler, Steven J. Price, Schyler O. Nunziata, Emily C. Moriarty Lemmon, Alan R. Lemmon, and David W. Weisrock

4:15 – 4:30 **Physiological Impacts of Simulated Climate Warming in the Hellbender**
Kimberly A. Terrell, Richard P. Quintero, Veronica Acosta, James B. Murphy, John D. Kleopfer, and Brian Gratwicke

4:30 – 5:00 Discussion and Wrap-up

6:00 – 7:00 p.m. Dinner (Grand Ballroom)

7:00 – 8:00 p.m. Posters & Dessert Social (Grand Ballroom)

8:00 - 10:00 p.m. Video Presentations (Grand Ballroom)

Wednesday, June 17 (Sheraton Clayton Plaza Hotel)

7:30 – 8:30 a.m. Continental Breakfast (Gallery Ballroom)

8:30 – 10:00 a.m. Oral Presentation Session (Gallery Ballroom)

Moderator: Jeff Ettling

8:30 – 8:45 **Effects of *Batrachochytrium dendrobatidis* on the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) Headstarting Program in Western New York State**

Joline Bruckman, Penny Felski, Nicole Dean, Kenneth Roblee, Greg Ecker, Dr. Kurt Volle, Dr. Robert J. Ossiboff, Dr. Elizabeth Bunting, and Julie Boerner

8:45 – 9:00 **Ranavirus Infection is a Threat to Cultured Chinese Giant Salamanders (*Andrias davidianus*) in China**

Yi Geng, Matthew J. Gray, Debra L. Miller, Xing Xing Liu, Yan Zhou, and Ze Hui Yu

9:00 – 9:15 **Exploration of the Range-wide Cutaneous Microbiome of the Eastern Hellbender**

Obed Hernandez-Gomez and Rod Williams

9:15 – 9:30 **Hematological and Immunological Characteristics of Eastern Hellbenders Parasitized by Leeches and Trypanosomes**

William A. Hopkins, Jesse Fallon, Michelle Beck, Brittney H. Coe, Catherine M.B. Jachowski, and Andrew Davis

9:30 – 9:45 **Pathogens vs. Pesticides: The Threat to Eastern Hellbenders**

William B. Sutton, Carson E. Lillard, Debra L. Miller, Reilly T. Jackson, Benjamin S. Wilson, Jennifer R. Asper, Rebecca P. Wilkes, Rachel D. Hill, Yi Geng, Bill Reeves, Dale McGinnity, Sherri Reinsch, and Matthew J. Gray

9:45 – 10:00 **Moving Forward in Understanding Disease of Wild Hellbenders**

Rebecca Hardman, W. B. Sutton, D. McGinnity, K. J. Irwin, S. Reinsch, B. Fitzpatrick, P. Colclough, M. Souza, M. Freake, M. J. Gray, and D. L. Miller

10:00-10:30 a.m. Break (Gallery Ballroom)

10:30-12:00 p.m. Follow-up Discussions and Planning for 8th Hellbender Symposium (Gallery Ballroom)

ORAL PRESENTATION ABSTRACTS

Sunday, June 14 Keynote Address

Separating the Wheat from the Chaff: Evaluating the Knowing and Doing of Hellbender Recovery

Gregory Lipps (Program Manager, Amphibian & Reptile Conservation, The Ohio State University)

Interest in the conservation of the hellbender has risen considerably since the first Hellbender Symposium brought together a small group of individuals in Helen, Georgia in 2003. Projects focused on the hellbender have now occurred in every state within the species' range, and many agencies, zoos, and NGOs are allocating significant resources to conserve and restore populations. While investigating novel and interesting questions is at the heart of biological research, successful conservation and recovery efforts may be more limited by our lack of implementation than our lack of knowledge. A survey of those working with the hellbender was conducted to answer three questions: (1) What do we think is important? (2) What are we actually doing? and, (3) Is there a "knowing-doing gap" in hellbender conservation? The results of the survey provide a benchmark from which to measure changes in the perceived importance of different recovery actions, as well as efforts to shrink the gap and avoid an "implementation crisis" in hellbender conservation. Addressing some gaps – such as reducing pollution, conserving riparian forests, and enforcing environmental regulations – ultimately requires engaging landowners, industries, policy-makers, and the decisions that they make. This engagement is time-consuming and at times frustrating, but is preferable to having history judge our efforts as simply documenting the path of the hellbender's extinction.

Tuesday, June 16 Morning Session

Hellbender Monitoring and Recovery Efforts in Missouri

Jeffrey T. Briggler (Missouri Department of Conservation)

Due to the decline of Ozark and eastern hellbender populations in Missouri, increased survey efforts and investigations into their decline have occurred over the past decade. Some current recovery efforts involve monitoring populations, assessing abnormalities and diseases (i.e., *B.d, B.s. Ranavirus*), assessing sperm profiles, investigating genetics, assessing microbiome community, collecting of eggs from the wild in natural nest and by artificial nest boxes, releasing of captive-reared animals, and continuation of the captive propagation programs at the Ron Goellner Center for Hellbender Conservation, Saint Louis Zoo and Shepherd of the Hills Hatchery. Overview of some of these recovery efforts will be briefly discussed, as well as planning/mitigation efforts and other noteworthy highlights. With collaboration of the Ozark Hellbender Working Group considerable effort is underway to investigate the decline of the hellbender in the Ozark Highlands, and we will continue our comprehensive efforts to recovery hellbenders in the Ozark Highlands.

Aspects of Eastern Hellbender Natural History from North-Central Pennsylvania

Peter J. Petokas (Lycoming College)

I studied eastern hellbender (*Cryptobranchus a. alleganiensis*) populations in the Susquehanna River watershed from 2006-2014. Tagging with passive integrated transponders (PIT tags) revealed that some individuals made long distance movements, mostly upstream and mostly adult males, with the record distance being 10 km upstream by an adult female. Clustering of adults and juveniles beneath a single cover rock was noted on multiple occasions, including two instances of 10 large adults beneath a single, large cover rock, once in September during the breeding season and once in late July, well outside the breeding season. Multiple adults were also observed together in bedrock crevices while diving in deep water. Clustering by older juveniles and young adults was also

observed on multiple occasions, with as many as six together beneath one cover rock in fast current conditions. Anatomical abnormalities are evaluated and described. Food habitats are evaluated in relation to season, temperature, food availability, and hellbender size. Habitat selection is evaluated based on cover rock size and location, channel morphology, and hellbender size and gender. American eels (*Anguilla rostrata*) were observed beneath hellbender cover rocks for the first time in 2014, but in no case were eels and hellbenders seen beneath the same rock. American eels had long been absent from the upper Susquehanna due to the presence of multiple dams down river, but were recently re-introduced to the watershed by USGS to bolster mussel reproduction. The precipitous co-occurrence of eels and hellbenders raises the concern that eels may compete with hellbenders for food and cover, that eels may predate hellbender eggs, larvae and juveniles, and that the eels could potentially initiate a decline in an otherwise stable hellbender population.

Nine Years of Eastern Hellbender Research in the Susquehanna River Watershed: Status of Extant Populations

Peter J. Petokas (Lycoming College)

I surveyed eastern hellbender (*Cryptobranchus a. alleganiensis*) populations in the Susquehanna River watershed from 2006-2014. Geographic distribution of the hellbender, in the Susquehanna of New York and Pennsylvania, is now severely restricted and most historic sites are no longer occupied, or have just a few resident individuals, with little or no evidence of reproduction and recruitment. One watershed population was observed to decline precipitously during our first four seasons of fieldwork (2006-2009) and recent surveys have found only a few individuals in the watershed. Of the self-sustaining populations identified, analyses of capture data show that the populations have been stable across sample years, with no significant change in linear density. Analyses of population structure indicate regular recruitment and an adult population containing many young adults. Fertile eggs, gilled larvae, and young juveniles are also regularly found at most sites. An analysis of skin swabs for *Batrachochytrium dendrobatidis* indicates a 40.4% *B.d.* infection rate across all populations combined. In the summer of 2014, and with assistance of the Upper Susquehanna Coalition, I began a program of artificial nest box installation at several stable population locations. Although the nest boxes remained unoccupied, I successfully removed eggs from natural nests in the late fall of 2014 and transported them to the Bronx Zoo in New York for a nascent head-starting program.

Eighteen years of Natural History Observations of Hellbenders in the Appalachian Mountains: Nesting Habitat, Breeding Behavior, Growth, and the Conservation Costs of Lifting Rocks

Jeff Humphries (North Carolina Wildlife Resources Commission)

This talk will focus on the life history of hellbenders in West Virginia and North Carolina, based on observing their habits, breeding and nesting behavior, marking individuals over the long-term, and observing changes in habitat use following passive and intensive surveying techniques for the past 18 years. Some observations include hellbenders being diurnally active in some parts of their southern range, up to 13 adult hellbenders using a single, small rock for reproduction, and male hellbenders guarding their young well into the following summer after they were hatched. Growth rates in the southern part of the hellbender's range appear to be extremely slow upon reaching the age of maturity, suggesting this species may live much longer than previously thought. Knowledge of the age of adult hellbenders has clear conservation implications in streams and needs to be a focus of monitoring and recovery programs, though much more research is needed. Finally, nest rocks are an extremely valuable and limited resource for hellbenders in Appalachian streams. The same nest rock can be used by hellbenders for breeding and nesting for many years, sometimes guarded by the same male, and disruption of these rocks by researchers or the general public can be extremely detrimental to hellbender populations over the long-run. Nest rocks in many streams have been lifted by many presumably well-meaning researchers and/or enthusiasts, resulting in the loss of these extremely important habitats. Once lifted or displaced, no matter how careful they are "replaced," nesting rocks often are no longer suitable for breeding and nesting, and often get tumbled downstream following flooding events, leaving them useless to hellbenders. Additionally, preliminary research in West Virginia suggested that rock lifting to capture hellbenders resulted in shelter rocks not being suitable for

hellbenders after only one summer of intensive rock-lifting surveys. Should we re-think the benefits and consequences of continuing to study the conservation status of hellbender populations using disruptive techniques? Are we studying this species to death?

Searching for a Needle in a Haystack: Assessing the Distribution of Hellbenders in New York's Susquehanna River Drainage

Robin Foster (SUNY Buffalo State), Amy McMillan (SUNY Buffalo State), and Chris Larsen (SUNY Buffalo State)

In New York State, hellbenders are found only in the Allegheny and Susquehanna River drainages. While the Allegheny has been well-studied, few thorough and systematic surveys have been conducted to assess hellbender habitat and populations in the Susquehanna/Chemung region. The watershed is very large, containing over 12,000 linear miles of stream. Hellbenders have historically been known from only two tributaries and the mainstem Susquehanna River. Fewer than five animals were reported from the watershed since 2005, with no signs of reproduction in several decades. It has been posited that hellbenders may be functionally extirpated from the New York portion of the Susquehanna, and restoration efforts are in the planning stages. In order to assess the present distribution of hellbenders in the watershed, an integrated, multi-method approach was employed. First, a species distribution model was developed using Maxent software for a regional scale including all of New York and Pennsylvania. Tributary reaches with suitability values greater than 0.6 were identified as potential hellbender habitat. These reaches were visually surveyed to assess habitat quality and ranked based on features including rock availability, water clarity, and riparian land use. More than 100 potentially habitable sites were identified on 18 tributaries and the mainstem river. Fifty of these sites were selected based on habitat quality ranking and history of hellbender occurrence. A combination of environmental DNA sampling and field surveys were used to determine presence/absence at each site. Several sites believed to have been extirpated were shown to have remnant populations, and a male guarding a viable nest was discovered. This research demonstrates the importance of using an integrated approach that employs multiple detection strategies in areas where hellbender populations may be very small or where detection is particularly challenging due to environmental conditions.

Physiography is a Stronger Predictor of Hellbender Occupancy in Virginia than Land Use

Catherine M. Bodinof Jachowski (Virginia Tech), Joshua J. Millspaugh (University of Missouri), and William A. Hopkins (Virginia Tech)

A major challenge to conservation planning for the eastern hellbender (*Cryptobranchus alleganiensis*) is a lack of information regarding current distribution. Our objective was to investigate factors associated with occurrence and to predict the probability of hellbender occupancy across an understudied portion of the species' total range in southwestern Virginia. We conducted snorkeling surveys in 49 stream reaches stratified across a land use gradient and two major drainages. We surveyed each reach on three occasions in either 2013 or 2014. We used Bayesian occupancy models to examine support for competing hypotheses regarding factors that influence hellbender occurrence. Specifically, we investigated support for our hypothesis that agriculture and urbanization negatively influence the probability of occupancy. Contrary to our predictions, hellbender occurrence was driven primarily by physiography, followed by land use quantified at the catchment scale. Occupancy was higher in reaches with catchments occurring primarily in the Blue Ridge relative to the Ridge and Valley physiographic province, regardless of major drainage or surrounding land use. In the Blue Ridge, occupancy was negatively associated with agriculture and urbanization, while occupancy in the Ridge and Valley was extremely low regardless of land use. Best performing models predicted that approximately 30% of our study area was occupied by hellbenders in 2013-2014. Our study is the first to predict hellbender occurrence across multiple drainages and highlights the potential for physiography to overwhelm land use effects over broad spatial scales. Our findings suggest that hellbender occurrence has the potential to be grossly over- or underestimated when physiography is unaccounted for, and we urge caution when attempting to predict hellbender occurrence based solely on commonly accepted species-habitat associations (e.g., heavily forested catchments).

Forest Removal: A Major Factor in the Decline of Hellbender Populations

Max A. Nickerson (Florida Museum of Natural History), Amber L. Pitt (Bloomsburg University), and Joseph J. Tavano (Bloomsburg University)

The habitat and population of Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) in North Fork of White River (NFWR), MO has been studied continuously since 1968. We review NFWR deforestation history in context of environmental changes. As forests were removed, especially riparian and nearby upland forests, a decades-long cascade began for Ozark hellbender habitat and populations in NFWR. Clearing for boat ramps, recreational facilities, airplane strips, canoe ranches, new bridges, homes, and agriculture exposed thin soil, limestone, dolomite, and sandstone. The lithological breakdown of these components produced silt, sand, chert, and a massive amount of small calcareous particles which washed into the river. These fine particulates filled interstitial spaces in the chert-gravel stream bottom, important habitat for hellbender larvae and macro-invertebrate prey. Record flooding washed the particles into NFWR until all of our known and suspected *C. a. bishopi* nesting sites were covered. Some formerly deep pool areas (to 2.5m) were filled and covered with small particulate material. A vast amount of material covered extensive sections of underwater ledges and rocky bottoms which served as adult hellbender diurnal sites. Riparian and upland land clearing provided greater access for recreational enjoyment and made the area a desirable location for businesses and people wishing to relocate. Unfortunately, the result has been increased clearing, pollution, coliform bacteria (including *E. coli*), green algae, rooted aquatic vegetation, siltation, introductions of hatchery water, and potential disruption of wildlife biology. Certainly the current habitat condition should greatly reduce the carrying capacity of NFWR Ozark hellbender populations when compared to past decades.

Nest-guarding Behavior of a Male Ozark Hellbender in a Natural Habitat

Rachel Settle (Missouri State University), Jeff Briggler (Missouri Department of Conservation), and Alicia Mathis (Missouri State University)

Male hellbenders engage in nest-guarding, resulting in enhanced egg and juvenile survival. In the wild, lack of juvenile recruitment into existing populations is a key factor in population declines, but the cause of low recruitment is unknown. As a part of population recovery efforts, the Missouri Department of Conservation collected video footage from a naturally-occurring nest of a single male Ozark Hellbender in the North Fork of the White River during the 2008 breeding season. We conducted a systematic analysis of the video footage to better understand nest guarding behavior of males. We quantified the following hellbender behaviors: tail-fanning, rocking, foraging, presence/absence at the nest, and egg cannibalism. We also recorded the presence of potential predators and prey. Overall, we observed high frequencies of tail-fanning and rocking, with tail-fanning occurring more frequently. These behaviors indicate that availability of oxygen is relatively low in the nest, suggesting that environmental perturbations (sedimentation, increased temperature) that further reduce oxygen levels could be of particular concern. The hellbender rarely left the nest unguarded and spent over half of the time exposed at the front of the nest. The number of eggs consumed by fish predators was low. Potential egg predators included centrarchid, cyprinid, ictalurid, and percid fishes, with centrarchids being the most common and also exhibiting the most interest in the nest. Although sculpin are common in this area, none were seen near the nest. The frequency of foraging by the male at the nest was low (8 total bites at prey), and over half of the foraging attempts at the nest were unsuccessful; the most frequent prey items consumed were small cyprinid fishes.

The Breeding Migration and Home Range Size of the Japanese Giant Salamander, *Andrias japonicas*, in a Headwater Stream with Special Reference to Den-masters

Sumio Okada (Japan Hanzaki Institute), Tamami Okada (Hanzaki Dojo), and Zach Felix (Reinhardt University)

It is commonly thought that *Andrias japonicus* migrate upstream into tributaries to breed. However, there is scant documentation on breeding migrations of *A. japonicus*. We conducted a radio-telemetric and mark-recapture study of *A. japonicus* to elucidate their migration patterns in a headwater stream of the Hino River, Tottori Prefecture, Japan. We succeeded in recording pre- and post-breeding migrations of five den-masters through

2002-2005. These are the first continuously recorded breeding migrations of *A. japonicus* by radio-telemetry. We compared home range size and migration patterns of den-masters with satellite males and gravid females.

Growth of a Long-lived Salamander in the Field: Over the 40 Years Study of More than 900 Identified Japanese Giant Salamanders, *Andrias japonicus* in Hyogo Prefecture

Yuki Taguchi (Japan Hanzaki Institute) and Takeyoshi Tochimoto (Japan Hanzaki Institute）

Accurate calculation "How long-lived species are growing" is challenging. When a big wild Japanese giant salamander (*Andrias japonicus*) is found, we are often asked "How old is this?" However, we didn't answer the question exactly. Because a giant salamander has been kept alive about 51 years in captivity and we know their growth rate is not consistence in different captive feeding conditions. Over the past 40 years we continued to survey *A. japonicus* in Ichikawa-river around Hanzaki Institute, Hyogo prefecture. We identified more than 1600 salamanders by photos and 900 by PIT tags. In this presentation we estimate the growth of *A. japonicus* by analyzing a long and large amount of data. We picked up 337 individuals with interval of captures was more than 5 years. Their total lengths were 145 to 1105, mean±SE was 688±8, and median was 690 mm. Growth rate were 42.2 to -9.4, mean±SE was 5.4±0.4, and median was 3.8 mm/year. It comes down gradually as they get older, which are divided mainly 3 terms by the lengths: 200-, 500-, 700-1100 mm. Moreover not a few animals became smaller and smaller. Now an individual of the longest interval of captures has grew 690 - 860 mm for 38.4 years. How old is this Japanese giant salamander?

The Use of Nest Boxes by the Hellbender Salamander in Western North Carolina

Arianne Messerman (University of Missouri), J.J. Apodaca (Warren Wilson College), Lori Williams (North Carolina Wildlife Resources Commission), Mark Endries (U.S. Fish and Wildlife Service), and Benjamin Prater (Wild South)

The hellbender salamander (*Cryptobranchus alleganiensis*) is a unique, large-bodied amphibian that serves as an excellent water quality indicator species in Western North Carolina. This animal has suffered substantial population declines over the past four decades throughout its range. Increased stream siltation largely attributed to human development fills the concave undersides of large rocks, consequently destroying hellbender breeding habitat. Habitat degradation has contributed to reductions in North Carolinian populations to such a degree that the species is now considered of Special Concern in the state. To restore hellbender population sizes under current land use conditions, researchers have recently begun developing artificial nest boxes that exclude sediment and promote increased reproduction. To identify the short-term efficacy of these shelters as substitutes for natural hellbender habitat in Western North Carolina, we constructed and placed 54 nest boxes across five river sites throughout the region in the summer of 2013. Following nest box installment, we examined each shelter through the breeding seasons of 2013 and 2014 for hellbender inhabitation and to determine the quality of water passing through the structures. Additionally, we created a maximum entropy species distribution model and conducted a spatial connectivity analysis for the hellbenders of Western North Carolina to identify ideal locations for nest box installation in the future. We detected hellbenders in the artificial shelters in one study stream in the summer of 2014. Additional structural improvements and time may reveal nest boxes to be useful conservation tools for this iconic species of Special Concern.

Hydrodynamic Redesign of Hellbender Salamander Nest Boxes

Mohammed G. Mohammed (University of Missouri), Kathleen M. Trauth (University of Missouri), and Arianne F. Messerman (University of Missouri)

The hellbender salamander (*Cryptobranchus alleganiensis*) has been designated a Species of Special Concern in North Carolina, and is in decline across its range. Breeding habitat loss due to increased sediment loads in streams is believed to be an important contributor to population declines. In order to address the loss of habitat, conservationists have begun deploying generally rectangular, sharp-edged nest boxes as artificial shelters. While data show that males have begun to use nest boxes for breeding in North Carolina, there remain two physical

difficulties with the model currently being used by the state. The first of these is the stability of nest boxes in flowing water, as current nest boxes have been found to move, even when rocks were placed on top of the structures. The second issue is that of the deposition of sediment at the down-stream face of the nest box, potentially blocking the entrance. The nest box currently in use has been redesigned consistent with hydrodynamic principles to address these issues. A curved upstream face (similar to the bow of a ship) has been introduced to reduce the drag force exerted by the flowing water. On the downstream side of the structure, the nest box is tapered to reduce the production and impact of opposing eddy currents that may result in sedimentation. In order to promote the tapering, the entranceway has been subsumed into the body of the nest box. Within the hydrodynamically efficient design, the dimensions have been specified to fit the nesting needs of the salamanders. Additionally, the weight of the structure has been limited to allow it to be carried into the field for placement. The theoretical design will need to be constructed and deployed in the field to test its performance in creating hellbender habitat.

Tuesday, June 16 Afternoon Session

Propagation Efforts for the Endangered Hellbender at the Saint Louis Zoo

Jeffery A. Ettling (Saint Louis Zoo) and Jeffrey T. Briggler (Missouri Department of Conservation)

The captive propagation and head-start program for hellbenders, *Cryptobranchus alleganiensis* was initiated by the Saint Louis Zoo and Missouri Department of Conservation in 2002. It has since grown to include other collaborators including the U. S. Fish & Wildlife Service, U.S. Forest Service, National Park Service and Arkansas Game & Fish Commission. The program was designed to provide stock for increasing wild population sizes through augmentation and if necessary reintroduction. While recruitment in the wild is low, some egg clutches have been discovered annually in recent years. Portions of these clutches have been brought to the Saint Louis Zoo for head-start and future release. The larger goal was to provide juveniles for release through captive reproduction, a feat that had never been achieved by any institution before. To help reach the goals of the program the Saint Louis Zoo constructed three environmentally-controlled rooms, two outdoor streams and has dedicated three full-time and one half-time keeper staff to this conservation propagation program. Since 2007 we have had Ozark hellbender, *C. a. bishopi* eggs laid every year, but it wasn't until 2011 that successful fertilization of the eggs occurred resulting in 185 larvae. To date Ozark hellbenders have successful bred for the past four years producing a total of 17 clutches of eggs. Currently more than 4,000 larvae are being reared from both captive-bred animals and eggs collected from the wild. The key to our success appears to be a combination of adequate space, appropriate sex ratios of animals, mimicking of environmental conditions, secure nesting areas and maintenance of high standards of water quality. Currently, we are expanding our captive-breeding efforts to Missouri populations of the eastern hellbender, *C. a. alleganiensis*.

Egg Incubation and Early Stage Larval Rearing of Hellbenders

Chawna Schuette (Saint Louis Zoo)

Since the onset of our captive propagation efforts for hellbenders, significant progress has been made at the Saint Louis Zoo in hatching hellbender eggs and rearing larvae. This presentation will highlight the techniques that have been developed and improved upon at the Ron Goellner Center for Hellbender Conservation. Through the incubation and hatching of over 5,000 hellbender eggs, both from wild collected and from those resulting from captive-breeding, a variety of abnormalities were observed and population demographic variables (e.g., growth rates, survivorship rates, etc.) noted. Also, different methods of incubation, fungicide, flow, dissolved oxygen, and techniques have been evaluated and will be discussed. The refinement in these techniques has resulted in increased success in egg hatching and survival of larvae.

Response to a Limited Feeding Regime in the Japanese Giant Salamander, *Andrias japonicus* at the Conservation Breeding Center, Asa Zoo, Hiroshima

Yuki Taguchi (Hiroshima City Asa Zoological Park) and Noriyuki Nonoue (Hiroshima City Asa Zoological Park)

Quite a few zoos and aquariums around the world rear giant salamanders. For their optimal health, it is important to know their feeding requirements. We investigated the feeding response of 14 Japanese giant salamanders (*Andrias japonicus*) with total lengths from 505 mm to 780 mm at the Conservation Breeding Center, Asa Zoo, Hiroshima. Salamanders were given just 1 % of their body mass of live fish (loach) every 10 days for 2 years. As a result of this experimental feeding their body mass increased by 2 to 6 % per year even at the low feed rate of 0.1 % of body mass per day. Their seasonal metabolism is also clarified: body mass increases in winter and decreases in summer if the same quantity of feed is offered year round. This seasonal response was affected by water temperature. The quantity of feed of 0.1 % of body mass per day is enough to maintain their body mass. We should design the feeding regime for giant salamanders in reference to not only feed volume but also their seasonal metabolism.

Captive-release Hellbender (*Cryptobranchus alleganiensis alleganiensis*) Movement in Two Northern Allegheny Streams

Julie A. Boerner (SUNY Buffalo State), Amy M. McMillan (SUNY Buffalo State), Robert J. Warren, II (SUNY Buffalo State), and Kenneth J. Roblee (SUNY Buffalo State)

Relocation, repatriation, and translocation (RRT) programs, including captive-release programs, have been used to promote species longevity. The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a species that may benefit from such programs. Eastern hellbender populations have recently declined throughout much of the animal's range. Some previous captive-release headstarting programs have resulted in minimal success, presumably due to movement of captive-released animals away from the release site containing suitable habitat and into areas of poorer quality. In addition, movement may lead to higher rates of predation. This study investigated movement of captive-released eastern hellbenders using three release methods: directly under a cover rock, held for a week in a nest box, and held for a longer period in a cage. Releases were conducted in two streams in the Allegheny River drainage. There was little difference in total distance moved and survivorship between stream sites or release treatments. Movement was most dependent on the phase of the moon. Both the distance and frequency of movements increased with a greater moon phase (closer to a full moon). Captive-released animals generally moved further than what has been reported in wild hellbenders, with an average cumulative distance moved of 653±138 m (SE). Overall survival was low; only three animals were found alive for longer than six months. Movement during periods of high moon phase does not seem to fit what is known about animal movement. Further studies need to be done to assess the effect of the lunar cycle on movement in wild hellbenders in comparison to captive-reared hellbenders. This could have important implications for hellbender conservation, such as how animals should be raised in captivity and when they should be released into the wild.

Survival, Movement, and Shelter Selection of Reintroduced and Translocated Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in West Virginia Following Rearing in Human Care

Joe Greathouse (The Wilds) and Eugene Felton (West Virginia University)

West Virginia was historically one of the states with the greatest distribution of hellbenders within the species' range. However, three survey efforts by different researchers over the past decade have indicated the presence of the hellbender in only 12 of 24 historic sites. We created models in MaxEnt and performed non-metric multidimensional scaling analyses to select a translocation site for head-started eastern hellbenders where they had once occurred but were no longer observed via physical or eDNA surveys that was most similar in landscape and in-stream attributes to sites where hellbenders were still observed in abundance.

We released 29 head-started hellbenders at a reintroduction site where the head-started hellbender eggs were collected as well as at the translocation site where hellbenders were no longer observed. Survival rates through 6 months during this study did not differ statistically between the translocation (60.0%) and reintroduction sites

(42.9%). The greatest causes of known mortality were predation by fur-bearers (80.0%) and burial under gravel and sediment during flooding (20.0%).

Movement distances and both linear and 95% minimum convex polygon home ranges were greater at the reintroduction site, and stream bank shelters were used more frequently at the reintroduction site. Increased movement and alternative shelter use are believed to be due to reduced shelter availability due to fewer boulders at the reintroduction site as opposed to conditions at the translocation site as well as the presence of adult hellbenders utilizing boulders at the reintroduction site.

Future hellbender conservation efforts in West Virginia should include continued reintroductions and translocations of head-started juveniles or sub-adults at appropriate sites with long-term monitoring for detection of sustainable populations as results of releases. Reintroduction efforts at sites with reduced boulder availability should also incorporate the utilization of in-stream restoration techniques such as the addition of boulders or concrete nest boxes.

Post-release survival of 31 Reintroduced Captive-reared Chinese Giant salamanders (*Andrias davidianus*)

L. Zhang (The Memphis Zoo, Mississippi State University, and The Shaanxi Institute of Zoology) W. Jiang (The Shaanxi Institute of Zoology), H. Zhang (The Shaanxi Institute of Zoology), Q. Wang (The Shaanxi Institute of Zoology), H. Zhao (The Shaanxi Institute of Zoology), R. Marcec (Mississippi State University and The Memphis Zoo), S. Willard (Mississippi State University), and A. Kouba (The Memphis Zoo)

The Chinese giant salamander (*Andrias davidianus*) has suffered an 80% population decline since the 1950s due to habitat destruction, water pollution, and over-harvesting. Few studies on reintroduction of captive-reared Chinese giant salamanders and monitoring of post-release survival have been reported. Reintroduction of captive-reared Chinese giant salamanders may augment the declining wild populations of this critically endangered species. Thirty-one juvenile salamanders were surgically implanted with radiotransmitters and were released at 2 sites in the Qinling Mountains of central China: the Heihe River site (animals: 3 years old, n = 15), and the Donghe River site (animals: 5 years old, n =16). Animal survival was monitored from April 2013 to July 2014. At the termination of the study, 2 of the animals released at the Heihe site were alive and traceable, while 10 were alive at the Donghe site. The status of other animals included: 9 were determined dead (29.0%), and 10 untraceable with status undetermined (32.3%). The annual survival rate of salamanders at Heihe was 0.593 (95% CI: 0.387 - 0.907), which was significantly lower than the rate at Donghe, which was 0.800 (95% CI: 0.621 - 1.000) ($\chi^2 = 3.6$, $P = 0.056$). Our results indicate that captive-reared giant salamanders are capable of survival in the wild.

How Population Genetics and Parentage Analysis Can Inform Headstarting Efforts

Amy McMillan (SUNY Buffalo State), Meghan Jensen (SUNY Buffalo State), and Robin Foster (SUNY Buffalo State)

Hellbender populations are declining in New York State. The Allegheny drainage has experienced a decline as great at 40% in the last 20 years and the Susquehanna drainage in NY may be functionally extirpated. As a result, the New York State Department of Environmental Conservation worked with the Buffalo Zoo to headstart hellbenders and have released many of these in the Allegheny. Headstart efforts also have begun for the Susquehanna with the Bronx Zoo. In this study we seek to understand the genetic structure and diversity of hellbenders in this region as well as determine parentage of the headstart cohort. Previous work in my lab on the Allegheny drainage showed that the headstart cohort was genetically less diverse than populations from throughout New York and Pennsylvania. A parentage analysis suggests that these headstart animals, although all related, arose from a mixed and large set of parents (up to seven mothers and seven fathers). Collaboration with Peter Petokas provided 40 animals from two sites in the Pennsylvania Susquehanna. Genetic analysis shows these animals are significantly less genetically diverse than even the headstart cohort, and much less diverse than the Allegheny drainage overall. These analyses will assist headstart initiatives by informing hellbender placement for genetic diversity goals in both drainages. I would like to have audience input into what those goals should be during a discussion following my talk and use this as a springboard for discussion of headstart initiatives and goals throughout North America.

Update on Environmental DNA Monitoring for Hellbenders in Georgia, North Carolina, and Tennessee

Stephen Spear (The Orianne Society), Michael Freake (Lee University), Lori Williams (North Carolina Wildlife Resources Commission), John Groves (North Carolina Zoological Park), Thomas Floyd (Georgia Department of Natural Resources), Christopher Jenkins (The Orianne Society), and Lisette Waits (University of Idaho)

Environmental DNA (eDNA) has provided a non-invasive method to detect species presence and has the potential to provide information on population size and other demographic parameters. As a result, there has been great interest in incorporating this technique into inventory and monitoring programs for threatened aquatic species. We have used eDNA to complement survey effort for eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*), and have demonstrated both its suitability for presence/absence as well as an increase in eDNA concentration during the breeding season. Here we report results of eDNA analysis from sites in Georgia, North Carolina, and Tennessee across 2013-2014. There was a general lack of eDNA detections across middle Tennessee, supporting the observations of declines in this region. In general, rate of eDNA detection was high in eastern Tennessee, North Carolina, and Georgia, although there were areas without eDNA positives in these regions. Similar to previous results in North Carolina, we did not see a significant association with eDNA concentration and snorkel survey numbers in Georgia, although this may be mostly related to variation in snorkel survey abundances. We also present preliminary information regarding the association of eDNA concentrations with eggs and larvae in both captive and wild hellbender environments.

Discovery and Utility of Sex-linked Genes in Cryptobranchid Salamanders

Paul M. Hime (University of Kentucky), Schyler O. Nunziata (University of Kentucky), Jeffrey T. Briggler (Missouri Department of Conservation), Joshua S. Reece (Valdosta State College), Alan R. Lemmon (Florida State University), and David W. Weisrock (Florida State University)

The ability to accurately determine sex is important for a wide range of basic and applied research questions. However, cryptobranchid salamanders exhibit limited sexual dimorphism, and distinguishing males from females can be extremely difficult outside of the breeding season, especially for young or non-reproductive individuals. Existing methods for determining sex such as ultrasound, hormone assays, or examination of external genital morphology can be inaccurate, and more invasive methods such as laproscopy or karyotype analysis may also be undesirable. Accordingly, we set out to identify sex-linked genes in *Cryptobranchus* and to leverage these sex-specific markers as a genetic sex assay. We used reduced representation genome sequencing by ddRAD-seq to identify sex-linked regions of the hellbender genome and to develop a robust PCR-based diagnostic for sex. We assessed the effectiveness of this assay in a series of blind trials across several divergent populations of hellbenders and found it to be robust across all *Cryptobranchus* populations surveyed and in both species of *Andrias*. Our results corroborate earlier cytogenetic research which suggested a ZZ/ZW sex determination system in cryptobranchids and further imply that female heterogamety may be the ancestral condition among Caudata. We anticipate that this genetic sex assay will have important implications for cryptobranchid research and conservation by enabling more informed captive breeding programs, permitting inclusion of sex as a variable for all age classes in ecological and demographic studies, and by facilitating investigation into sex-biased patterns of dispersal within and between populations.

Genome-scale Resolution of Species Boundaries and Demography in *Cryptobranchus*

Paul M. Hime (University of Kentucky), Jeffrey T. Briggler (Missouri Department of Conservation), Steven J. Price (University of Kentucky), Schyler O. Nunziata (University of Kentucky), Emily C. Moriarty Lemmon (Florida State University), Alan R. Lemmon (Florida State University), and David W. Weisrock (University of Kentucky)

In this talk, I will describe our progress in developing a diverse suite of genomic resources in cryptobranchid salamanders and our ongoing efforts to leverage these tools towards a comprehensive understanding of the evolutionary and demographic history of *Cryptobranchus*, with an emphasis on "eastern" and "Ozark" populations in Missouri. Several recent studies have successfully exploited mtDNA sequencing and microsatellite markers to reveal significant patterns of population genetic differentiation across the range of the hellbender, hinting at the

possibility of multiple divergent lineages. Yet, species boundaries in *Cryptobranchus* (if any) remain poorly understood, and given the significant riverine isolation between different populations, the potential may exist for undescribed, cryptic species-level diversity. To obtain robust estimates of key population genetic and demographic parameters, and to rigorously assess the potential species status of these divergent hellbender lineages under the biological species concept, we leverage phased sequence data from ~55,000 single-copy nuclear loci in an explicit coalescent framework. We reveal that genome-wide levels of heterozygosity vary significantly between rivers and also provide new perspectives on population genetic differentiation between populations. Importantly, we also utilize comparative outgroup sequence data from both species of *Andrias*, and two independent methods for coalescent species tree estimation and a multilocus genealogical analysis are all concordant in recovering clear and strongly supported patterns of lineage relationships. We next rigorously evaluate the potential that divergent hellbender lineages represent distinct species. Additionally, we implement a new method for demographic model selection and parameter estimation to obtain estimates of divergence times between lineages, rates and directions of historical and contemporary gene flow, and effective population size fluctuations through time. We expect that this work will be informative for conservation efforts in *Cryptobranchus* and that these genomic resources will have utility in a wide range of research questions across the family Cryptobranchidae.

Physiological Impacts of Simulated Climate Warming in the Hellbender

Kimberly A. Terrell (Smithsonian Conservation Biology Institute), Richard P. Quintero (Smithsonian's National Zoological Park), Veronica Acosta (Smithsonian's National Zoological Park), James B. Murphy (Smithsonian's National Zoological Park), John D. Kleopfer (Virginia Department of Game and Inland Fisheries), and Brian Gratwicke (Smithsonian Conservation Biology Institute)

Climate change is expected to become a major driver of extinction among cold-adapted salamanders, including the hellbender (*Cryptobranchus alleganiensis*). Increased knowledge of salamander thermal physiology is needed to help understand and mitigate the consequences of a warmer climate. We investigated the physiological effects of seasonal temperature variation and moderate (2°C) warming in a captive population of juvenile eastern hellbenders (*C. a. alleganiensis*). Specifically, we predicted that exposure to current peak summer temperatures would stunt growth, decrease innate immune function, and increase physiological stress. We further predicted that these impacts would be exacerbated in hellbenders exposed to simulated warming. Experimental treatments were based on water temperatures recorded hourly from Aug 2011 – Aug 2012 at the site where hellbenders were collected as embryos (Allegheny River, NY). Hellbenders (n = 6 per group) were exposed to current temperature patterns (i.e., based on 2011-2012 data) or 2°C warmer values. A control group (n = 6) was maintained at a constant mean temperature (15°C). Exposure to peak summer temperatures (representing the current climate) caused a 7% monthly decline in body mass. Unexpectedly, a (9%) decline in body mass was also observed during exposure to current winter temperatures. Compared to individuals exposed to a current climate, warmer (2°C) summer temperatures further reduced growth, decreased immune function, and increased physiological stress ($p < 0.05$). In contrast, warmer (2°C) winter temperatures mitigated the decline in body mass associated with cold exposure ($p < 0.05$). These findings highlight the need to consider the sub-lethal impacts of warmer summers and the potential mitigating effects of milder winters in predicting climate change impacts in hellbenders and other cold-adapted salamanders.

Wednesday, June 17 Morning Session

Effects of *Batrachochytrium dendrobatidis* on the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) Headstarting Program in Western New York State

Joline Bruckman (New York Department of Environmental Conservation), Penny Felski (The Buffalo Zoo), Nicole Dean (Cornell University- Animal Health Diagnostic Center), Kenneth Roblee (New York Department of Environmental Conservation), Greg Ecker (New York Department of Environmental Conservation), Dr. Kurt Volle (The Buffalo Zoo), Dr. Robert J. Ossiboff (Cornell University), Dr. Elizabeth Bunting (Cornell University), and Julie Boerner (SUNY Buffalo State)

From 2011-2013, 359 eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) reared in captivity from wild caught egg masses have been released in the Allegheny River drainage as a part of the New York State Department of Environmental Conservation's hellbender headstarting program in conjunction with The Buffalo Zoo. Monitoring of these hellbenders indicated an estimated survival rate of only 4-8%, with low fidelity to release sites and substantial downstream movement. In order to determine if predation associated with hellbender movement was contributing to low survival, a soft release telemetry study of 18 hellbenders was initiated in 2013. During the study 14 hellbenders perished, while 4 went missing. One recovered carcass showed no signs of predation, but skin swabs revealed high loads of *Batrachochytrium dendrobatidis* (*Bd*) fungus. In 2014, to better assess the role of *Bd*, hellbenders were released individually into submerged cages that permitted regular observation, skin swabbing, and morphometric data collection. Within 4-8 weeks, monitoring showed that of the 25 hellbenders released, most were either found lethargic with abnormal skin coloration or recently deceased. In conjunction with the Wildlife Health Program at Cornell University, quantitative PCR (qPCR) testing of skin swabs revealed that sick hellbenders were strongly positive for *Bd*. By the end of the 2014 field season, 19 of the 25 (76%) hellbenders had died, and postmortem examination revealed changes consistent with death due to chytridiomycosis. While wild hellbenders are often infected with low loads of *Bd* that are unlikely to be of clinical significance, chytridiomycosis was the major cause of mortality in headstarted hellbenders released in 2014, and is suspected to be a significant cause of the overall poor survival in previous years. These findings illustrate an important potential obstacle for hellbender headstarting programs.

Ranavirus Infection is a Threat to Cultured Chinese Giant Salamanders (*Andrias davidianus*) in China

Yi Geng (Sichuan Agricultural University, University of Tennessee), Matthew J. Gray (University of Tennessee), Debra L. Miller (University of Tennessee), Xing Xing Liu (Sichuan Agricultural University), Yan Zhou (Sichuan Agricultural University), and Ze Hui Yu (Sichuan Agricultural University)

The Chinese giant salamander (*Andrias davidianus*), which belongs to the order Caudata and family *Cryptobranchidae*, is an endangered endemic amphibian in China. In the past 30 years, artificial breeding and farming has been used to protect Chinese giant salamanders. Recently, Chinese giant salamander ranavirus (CGSRV) was identified as a novel pathogen of Chinese giant salamanders, causing mass morbidities and mortalities, which has severely impacted the Chinese giant salamander breeding industry. Since CGSRV infection was discovered in 2009 in Shanxi Province, at least 11 provinces of China have reported and confirmed this disease. Economic losses have exceeded millions in USD. Duration from infection of CGSRV to death is 15 – 40 days. Mortality rate is typically 70 – 90% in affected larvae and 30 – 45% in adults. Ubiquitous bacteria, such as *Aeromonas hydrophila* and *Aeromonas veronii*, can infect secondarily and increase mortality rates. Gross clinical signs include skin discoloration and erythema, anorexia, lethargy, bloody feces, vomiting, swelling of the legs, marked abdominal distension, and skin nodules and ulcerations. Occasionally, necrosis and loss occurred in the distal extremities. During necropsy, the most common lesions included petechial or ecchymotic hemorrhages of the internal organs, especially the livers, kidneys, lungs and spleens; pale swollen livers; and swollen kidneys and spleens. The objective of our presentation is to review the impact of CGSRV on the Chinese giant salamander, discuss the pathology of the pathogen, and summarize other advances in research, such as effective molecular

diagnostic techniques and options currently available for treatment. Lessons learned in China about CGSRV might be useful for planning conservation efforts in North America for *Cryptobranchus alleganiensis*.

Exploration of the Range-wide Cutaneous Microbiome of the Eastern Hellbender

Obed Hernandez-Gomez (Purdue University) and Rod Williams (Purdue University)

Culture-independent approaches, such as genetic sequencing of bacterial DNA, allow for the characterization of whole microbial communities within hosts. This technique provides an opportunity to identify beneficial symbionts, pathogens, and compare microbial communities of hosts across space and time. Community analysis of the microbiome of amphibians is important due to the current negative effects disease is imposing upon populations world-wide. Hellbenders have experienced range-wide population declines, and as a result conservation programs have utilized translocations/re-introductions to supplement decimated populations. However, with the known differences in health, genetic structure, and fragmentation across the species' range, the need to explore the microbiota of hellbenders is crucial maximize translocation effectiveness and prevent disease spread. Previous microbial characterization has illuminated the composition of the microbial communities on the skin and wounds of eastern and Ozark subspecies in Missouri. We expanded the existing knowledge of the hellbender cutaneous microbiome, by analyzing skin microbiome samples from across the range of the eastern subspecies (Indiana, West Virginia, Tennessee, Georgia, Virginia, and North Carolina). Sequencing returned more than 20.7 million reads, from which 5.7 million were used to identify 4,557 operational taxonomic units. Microbial community comparisons show patterns of differentiation across the range of the eastern hellbender, with the strongest differences noted at the drainage level. Common bacteria across the range include members of the phyla Proteobacteria, Actinobacteria, Cyanobacteria, and Firmicutes. The patterns of microbiome differentiation observed across the range of hellbenders support the contemporary use of genetics as a translocation guide, indicating a low risk of pathogen transmission associated with within drainage movement of animals.

Hematological and Immunological Characteristics of Eastern Hellbenders Parasitized by Leeches and Trypanosomes

William A. Hopkins (Virginia Tech), Jesse Fallon (Virginia Tech), Michelle Beck (Virginia Tech), Brittney H. Coe (Virginia Tech), Catherine M.B. Jachowski (Virginia Tech), and Andrew Davis (University of Georgia)

Disease is among the factors implicated in hellbender population declines, but the responses of hellbenders to parasites and pathogens remain poorly studied. We recently discovered a new species of leech that parasitizes hellbenders in Virginia, and experimentally demonstrated that it disrupts important hellbender hormonal pathways and transmits blood parasites (trypanosomes) to hellbenders. Here, we document the prevalence of leech and trypanosome parasitism and co-parasitism in a population of eastern hellbenders and describe hematological and immunological characteristics of hellbenders harboring parasites. We hypothesized that hellbenders parasitized by trypanosomes would be anemic, that individuals parasitized by either organism would exhibit shifts in white blood cell counts, and that hellbenders harboring leeches would exhibit altered plasma bactericidal capacity. We found that 24% and 68% of hellbenders in our sample population were parasitized by leeches and trypanosomes, respectively. We found no evidence suggestive of anemia. However, we found that hellbenders with either parasite exhibited marked shifts in circulating white blood cells that were consistent with adaptive responses to parasitism (i.e., increased % neutrophils, decreased % lymphocytes, and increased % eosinophils). In addition, hellbenders harboring leeches had much higher plasma bactericidal capacity than individuals without leeches, and we offer potential mechanistic explanations for this observation. We also found strong evidence, both *in vivo* and *in vitro*, that cellular and serological immune responses to parasites were less robust in juvenile than adult hellbenders. This finding warrants further investigation in light of the demographic characteristics, specifically the scarcity of juvenile age classes, of declining hellbender populations. Finally, we describe methodological advances that will improve future studies seeking to diagnose trypanosome parasitism in hellbenders. Taken together, our study provides fundamental insights into how hellbenders physiologically respond to parasites, which could ultimately prove useful for understanding circumstances where disease is a suspected contributor to population declines.

Pathogens vs. Pesticides: The Threat to Eastern Hellbenders

William B. Sutton (Tennessee State University, University of Tennessee), Carson E. Lillard (University of Tennessee), Debra L. Miller (University of Tennessee), Reilly T. Jackson (University of Tennessee), Benjamin S. Wilson (University of Tennessee), Jennifer R. Asper (University of Tennessee), Rebecca P. Wilkes (University of Tennessee), Rachel D. Hill (University of Tennessee), Yi Geng (University of Tennessee, Sichuan Agricultural University), Bill Reeves (Tennessee Wildlife Resources Agency), Dale McGinnity (Nashville Zoo), Sherri Reinsch (Nashville Zoo), and Matthew J. Gray (University of Tennessee)

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) of North America is declining in several watersheds across its geographic range. Frequently, these watersheds are degraded due to surrounding agricultural land use. The increase in application of glyphosate in the 1990s coincided with the decline of hellbenders in some watersheds. To date, no studies have tested the toxicity of glyphosate herbicides to hellbenders. The 1990s also were a period when several pathogens emerged in amphibian populations, but their effects on hellbenders remain unclear. We performed a series of experiments with Cornerstone® and Roundup® formulations of glyphosate in an attempt to identify the lethal concentration (LC) that $\geq$50% of 6-mo old hellbender larvae die. We also tested the susceptibility of hellbender larvae to ranavirus exposed to two temperatures (15 and 22 C). Of the concentrations tested (0.03, 0.3, 3, 6, 12, and 24 ppm), there was 100% mortality in three days for the 24-ppm Roundup® treatment; no other mortality occurred. Mortality was 67% for hellbender larvae exposed to ranavirus at 22 C; no mortality occurred at 15 C. Our results indicate that hellbender larvae can tolerate high concentrations of glyphosate herbicide, and Roundup® may be more toxic than Cornerstone®. The average LC_{90} for three *Ambystoma* species and eastern newt (*Notophthalmus viridescens*) larvae in a previous study was 7X lower (3.35 ppm) than what we found for larval hellbenders. It is rare for glyphosate concentrations in the water column of streams to exceed 10 ppm. The greater pathogenicity of ranavirus at 22 C is likely a consequence of faster viral replication, which occurs at higher temperatures. Land-use practices that contribute to increasing water temperatures in streams (e.g., shade tree removal, reducing flow) could trigger ranavirus outbreaks in hellbender populations. Future experiments include testing the pathogenicity of *Batrachochytrium dendrobatidis* (*Bd*) to juvenile hellbenders, and crossing pathogen treatments (*Bd* and ranavirus) with sublethal concentrations of glyphosate herbicides to test for interactive effects.

Moving Forward in Understanding Disease of Wild Hellbenders

Rebecca Hardman (University of Tennessee), W. B. Sutton (Tennessee State University), D. McGinnity (Nashville Zoo), K. J. Irwin (Arkansas Game and Fish Commission), S. Reinsch (Nashville Zoo), B. Fitzpatrick (University of Tennessee), P. Colclough (Knoxville Zoo), M. Souza (University of Tennessee), M. Freake (Lee University), M. J. Gray (University of Tennessee), and D. L. Miller (University of Tennessee)

The hellbender, *Cryptobranchus alleganiensis*, is a large aquatic salamander containing two subspecies (Ozark hellbender, *C. a. bishopi* and eastern hellbender, *C. a. alleganiensis*) from the Ozark mountains and eastern U.S., respectively. Both subspecies have seen population declines over the past 25 years, especially in *C. a. bishopi* which is federally endangered. Habitat degradation and possibly low genetic diversity may lead to secondary infections with amphibian pathogens such as *Ranavirus* and *Batrachochytrium dendrobatidis* (*Bd*). Other pathogens such as the emerging salamander chytrid (*Batrachochytrium salamandrivorans* or *Bsal*) and other bacterial or fungal species are also of concern as either primary or secondary causes of disease. Our objective is to determine prevalence of these pathogens in both subspecies to understand the role of emerging amphibian pathogens in *C. alleganiensis* declines. We collected tail tissue and skin swabs from *C. a. bishopi* and *C. a. alleganiensis* individuals from Arkansas and Tennessee respectively during the summers of 2011-2014. We used qPCR analysis to determine presence of *Ranavirus* and Bd from tail samples and skin swabs, respectively. In the latter two years we collected swabs for metagenomic analyses of active lesions. Overall, for *C. a. bishopi*, we detected 27% prevalence of *Bd* and no cases of ranaviral infections; for *C. a. alleganiensis,* we detected 15% prevalence of *Bd* and 3% prevalence of *Ranavirus*. We have not found any *Bsal* positive individuals. These data reveal that *Bd* is present in these populations. We are currently in our second phase of investigating morbidity and mortality in hellbenders. We are incorporating metagenomic comparisons of lesions and healthy skin, bacterial peptide production, and classification of chytrid strains potentially unique to wild hellbender populations.

POSTER ABSTRACTS

(Names in alphabetical order)

Response of Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) to Native and Exotic Prey

Zach Cava (SUNY Buffalo State)

The spread of non-indigenous species is a leading cause of animal extinctions and a primary agent of global biotic homogenization. By altering the structure and dynamics of native food webs, invasive species can initiate trophic cascades, which potentially simplify ecosystems, leading to reduced functionality, resilience, and stability. Consequently, elucidating how exotic species affect food-webs is critical to understanding the broader ecological consequences of species invasions and developing adaptive management strategies. Studies of predator-prey relationships in invasion biology have typically focused on the responses of exotic prey to native predators, and less is known about how predators respond to non-indigenous prey. Here I investigate the response of a rare and threatened native predator—the eastern hellbender (*Cryptobranchus alleganiensis*) to a high-profile invasive crayfish species, *Orconectes rusticus*.

Hellbenders have declined throughout much of their historic range, and the spread of non-native species is a factor implicated in its decline. While the potential for exotic predators (i.e. sport fish) to negatively impact *C. alleganiensis* has been addressed, little is known about how hellbenders may be affected by exotic prey. Crayfish are an important food resource for *C. alleganiensis*; however, some speculate that the large and aggressive *O. rusticus* may be unpalatable to hellbenders in regions where these two species have not historically co-occurred. Thus, the objectives of this study are: 1) to investigate the behavioral response of hellbenders to native vs. non-native crayfish, and 2) to use the resulting data to infer how crayfish population demography might influence hellbender abundance at the local scale.

Eastern Hellbenders get a Head-start at a Missouri Department of Conservation Hatchery

James A. Civiello (Missouri Department of Conservation) and Jeffrey T. Briggler (Missouri Department of Conservation)

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a large aquatic salamander of cool streams and rivers with moderate to swift-flowing current and large rocks. Populations are found in the Gasconade, Big Piney, Niangua, and Meramec rivers. Populations have declined by 80% since the 1970s and the population structure has shifted towards larger individuals. Consequently, the species was listed as critically imperiled and state endangered in 2003. Declining numbers of wild hellbenders have necessitated the use of captive propagation to ensure long-term recovery. To bolster populations, captive propagation has been used at the Saint Louis Zoo since 2002 and at MDC Shepherd of the Hills Hatchery since 2007. Approximately 1,800 eastern hellbenders have been hatched and reared at Shepherd of the Hills Hatchery since 2007 from eggs collected from the wild, and 341 individuals have been released into their river of origin. Captive propagation efforts are the "safety net" currently implemented to bolster existing populations while we continue to investigate the reasons for decline.

A Historical Approach to Investigating Declines of the Eastern Hellbender (*Cryptobranchus alleganiensis)* in New York and Pennsylvania

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When planning for conservation and management, lack of historical data often poses challenges for species that have not been intensively studied or collected in the past. One such challenge is the issue of shifting baselines, a phenomenon in which each generation views a progressively poorer resource base as normal. In the case of hellbenders (*Cryptobranchus alleganiensis*), few scientific studies were conducted on the species prior to the late

1970s or early 1980s, and although hellbenders were collected for various uses, official records of hellbender harvests were not kept. This has led to a dearth of information about historic distribution and relative abundance, making it difficult to determine the extent of hellbender declines or pinpoint when and why declines began. This study uses a variety of historical sources, including newspaper articles, natural history expedition records, and personal accounts to map the former distribution of hellbenders in New York and Pennsylvania. Information relating to potential causes of decline was also recorded. Preliminary results indicate that hellbenders historically had a restricted range with low relative abundance within the Upper Susquehanna watershed, while their range and numbers in the Pennsylvania Susquehanna expanded in the mid-1800's. In addition, this research revealed a history of negative attitudes toward the species and high frequency of hellbenders being killed by recreational stream users. This study will help to better elucidate the timing and pattern of hellbender declines in New York and Pennsylvania, and may lead to a better understanding of the factors that have driven this process.

Estimating the number of river miles with high hellbender densities using point surveys in Tennessee

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The Tennessee valley may have some of the best remaining hellbender populations, yet there is very little historical data to compare against current population structure and density. Moreover, it is not clear just how many river miles within the Tennessee Valley actually support healthy hellbender populations. It is tempting to extrapolate from studies at specific locations and assume that similar patterns extend up and downstream. We have been conducting point surveys on rivers in Tennessee using snorkeling and trapping methods, with a particular focus on rivers that flow through both public and private land. These rivers include Hiwassee, Little, Tellico, Doe and Beaverdam. We expected that habitat impairment would be more apparent on private land sections, resulting in lower quality habitat. We found that hellbender populations appear to decline substantially as rivers leave protected Forest Service and National Park lands, and even within public lands there can be significant variation in population density, possibly due to habitat variation and barriers to movement. Populations in the Tennessee valley are very fragmented, and in many cases very isolated from one another, with apparently healthy populations found in rather few river miles.

The Historic Distribution of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in the Susquehanna Drainage of Pennsylvania

Sean M. Hartzell (Bloomsburg University) and Amber L. Pitt (Bloomsburg University)

The eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is imperiled due to a variety of factors such as habitat degradation, disease, and collection. A challenge for assessing changes in hellbender populations and distribution is that historical records have not been compiled into a single database, in part because of the myriad sources of data and the time associated with finding and compiling records. Eastern hellbenders occur within the Susquehanna River drainage of the eastern United States and evidence suggests that hellbender populations are declining and distribution is decreasing in portions of the drainage. Historical records from the drainage are somewhat sparse and unconsolidated. In order to establish a baseline for comparison with future studies, we compiled historic accounts of hellbenders within the Susquehanna drainage of Pennsylvania. Historic accounts consisted of museum and survey records, government reports, atlases, newspaper and magazine articles, and reputable personal communications. Records dated back to the late 1800s and the most recent near drainage-wide survey was conducted in the early 1990s. Historically, hellbenders occupied a wide distribution within the Susquehanna Drainage of Pennsylvania, occurring in at least 24 streams as well as the Susquehanna River itself. Records suggest a historic range encompassing at least 18 counties. Distributional records from the Pennsylvania Reptile and Amphibian Survey (PARS) Database and the two published distributional volumes concerning Pennsylvania's herpetofauna report the historic occurrence of hellbenders within the Susquehanna drainage of Pennsylvania collectively within 13 counties and do not list specific tributary occurrence. Hence, this work expands the known historic distribution of the hellbender in the drainage.

Next Generation Sequencing Comparison of the Cutaneous Microbiome of Two Amphibian Subspecies in Relation to Health and Disease

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Technological advances in DNA sequencing and data analysis have resulted in a plethora of studies that characterize microbiomes within multiple environments. Characterization of microbial communities has become useful in studying the ties between organismal health and the host microbiome. Expanding this technique in wildlife studies has been slow, though disease threatens populations worldwide. Pathogens currently threaten the existence of many amphibian species, and some studies have characterized the microbiome on the skin of this class. Hellbender salamanders provide an ideal system to explore the relationship between microbiome and disease. The Ozark hellbender subspecies currently expresses chronic wounds believed to be caused by bacterial infections, whereas the eastern hellbender does not. We are the first to apply the use of high throughput sequencing to explore the potential differences in the skin bacterial community composition between these two amphibian subspecies. Through bacterial 16s rRNA amplicon sequencing we were able to detect 8,118 distinct operational taxonomic units. We found differences in the bacterial communities between the two subspecies, while there were no differences between the Ozark wound and healthy samples nor between respective subspecies environment samples. Several opportunistic pathogens were found to have an association with both Ozark healthy and wound samples. These findings suggest that wounds present only in the Ozark hellbenders may be due to a reduced immunocompetence relative to eastern hellbenders. Using next generation sequencing allowed for an in-depth exploration of the distribution and abundance of bacteria between two threatened amphibian subspecies.

Advancing Head-Starting Techniques for Eastern Hellbenders through Novel Environmental Conditioning

Erin K. Kenison (Purdue University) and Rod N. Williams (Purdue University)

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) was once common throughout the eastern United States, but is now threatened or endangered throughout most of its range. In Indiana, it is state-endangered and restricted to a single river system. There has been no evidence of recruitment for decades and older individuals make up the majority of the population, making extirpation a critical threat. Head-starting and reintroduction efforts are commonly used to supplement and conserve wild populations, however, released individuals must respond appropriately to predators, have robust immune systems, and forage successfully to survive. Although some behavioral and physiological responses are innate, larval hellbenders may require certain conditions that mimic the natural environment to develop appropriate responses to threats. We will conduct multiple experiments that will expose larvae to water current, predator cues, and the microbial community of natural river water. This is the first project to test novel head-starting methods and investigate the effects of environmental conditioning on larval morphology, behavior, stress, and immune response in a captive setting. Advancing captive rearing techniques may be the most feasible way to increase juvenile hellbender survival following reintroductions and augment remaining populations in Indiana. We strive to provide meaningful information to zoos to advance captive-rearing techniques and valuable insight to agencies to aid in hellbender management and conservation decisions throughout the nation.

A Multispecies Distribution Model for the Conservation and Management of Indiana's Eastern Hellbenders

Emily B. McCallen (Purdue University), Rod N. Williams (Purdue University), and Songlin Fei (Purdue University)

Eastern hellbender population densities have declined throughout their range. In Indiana, the eastern hellbender is now restricted to a single river system. Conservation efforts, which require explicit knowledge of hellbender habitat requirements, are currently underway to ensure continued population persistence. Previous studies of hellbenders have focused on microhabitat level variables to characterize habitat use. However, the practical applications of previous findings are limited due to the extensive effort needed to characterize microhabitat data at a relevant spatial scale. Such limitations may be overcome through the use of species distribution modeling

(SDM). The SDM process relates remotely sensed environmental variables to patterns of known species occurrence and results in a predictive map of habitat suitability covering a continuous area of interest. The predictive power of SDMs may be improved by accounting for biotic interactions. In this study, we will use SDM to predict the distribution of hellbender at 300-m spatial resolution with the consideration of biotic interactions in the Blue River system in Indiana. Field surveyed population data for eastern hellbenders, along with latrine survey based population data for northern river otters (*Lontra canadensis)*, a predator and competitor of hellbenders, will be used in our SDMs. Environmental variables used in our model include both in-stream characteristics, such as sinuosity, gradient, and substrate type, and landscape characteristics, such as land use type and canopy cover. Our SDM predictions will provide guidance for future hellbender sampling efforts and help determine optimal sites for hellbender translocations and reintroductions in Indiana.

Artificial Nest Boxes for Hellbender Egg Collection

Peter J. Petokas (Lycoming College), Christopher D. Yearick (Upper Susquehanna Coalition), and James Curatolo (Upper Susquehanna Coalition)

Population declines have been taking place throughout the entire geographic range of the eastern hellbender (*Cryptobranchus a. alleganiensis*) in North America. The declines and local extinctions have led to multiple head-starting programs to augment or restore hellbender populations. Once widespread throughout the Susquehanna River basin, the eastern hellbender is now restricted to several tributaries of Susquehanna River. To facilitate the collection of fertilized eggs for a nascent head-starting program for the Susquehanna watershed, we installed 17 artificial nest boxes in the summer of 2014. The boxes were made using fiberglass-reinforced concrete formed by hand over a wooden form. Four-inch PVC couplings were added to the top center and one side. The top fitting accepts a screw-in cap for easy access to the nest chamber. The side fitting accepts a four-inch section of PVC pipe to provide an elongate entryway for hellbender entry to the chamber. When the concrete has dried sufficiently, the form is knocked out to create a nest chamber. Although weighing a hefty 27 kg, the concrete boxes are easily carried or floated to installation sites. Each of the nest boxes was placed atop a clean, flat gravel bed. The concrete box was then covered with large boulders, leaving only the access tunnel visible. Despite our efforts to force occupancy by placing an adult male inside each chamber, none of the nest boxes remained occupied by hellbenders during the fall 2014 breeding season. Additional nest boxes will be constructed and installed in the summer of 2015, and we are transitioning to black PVC fittings and pipe to minimize visibility of the artificial nest boxes to human passersby.

Quantitative Analysis of Reproductive Behavior of the Ozark Hellbender in Captivity

Rachel Settle (Missouri State University), Chawna Schuette (Saint Louis Zoo), Jeff Ettling (Saint Louis Zoo), Mark Wanner (Saint Louis Zoo), Jeff Briggler (Missouri Department of Conservation), and Alicia Mathis (Missouri State University),

Kinematic diagrams illustrate transitions of behaviors that occur in sequences and allow for some predictability of recurring events. We constructed kinematic diagrams of behaviors before, during, and after successful oviposition events for captive Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*). A 4-camera recording system monitors an indoor artificial stream at the Saint Louis Zoo's Ron Goellner Center for Hellbender Conservation. Hellbender behavior was recorded between the hours of 20:00 and 08:00, which allows for playback of reproductive events and detailed analysis of behavior. We observed hellbender behaviors during the 2012 reproductive season and recorded the occurrence of aggression, sexual, and solitary/locomotion behaviors. Oviposition occurred on two different days of one week during this season. We designated specific periods for each kinematic diagram: Pre-oviposition, oviposition event A, inter-oviposition, oviposition event B, and post-oviposition. Overall, both general activity and aggressive behaviors increased substantially through the oviposition event A, peaked during inter-oviposition, and then declined abruptly following oviposition event B. Sexual behaviors tended to occur only on those days in which oviposition occurred. Two females were involved in oviposition event A, both of which deposited their eggs in the same nest box consecutively. A third female deposited her eggs in a separate nest box during oviposition event B. Our findings suggest that dramatic increases in activity during the breeding season indicate that oviposition is likely to be imminent (likely within a day).

Understanding behaviors that occur during the reproductive season can be used to make important management decisions and thus increase the success of captive breeding programs.

Reassessment of Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) Distribution in the Susquehanna River Drainage of Pennsylvania

Jamie L. Shinskie (Bloomsburg University) and Amber L. Pitt (Bloomsburg University)

The native distribution of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis)* extends from southern New York to northern Alabama with a disjunct population in central Missouri. Population declines have been documented throughout their native range, and it has been hypothesized that hellbenders have undergone substantial range constrictions due to population extirpations. However, data for many portions of their range are deficient for assessing this hypothesis. Hellbenders occur in the Susquehanna River drainage in the northeastern United States. With the exception of a few populations in the West Branch sub-basin, hellbender populations within the Susquehanna River drainage in Pennsylvania have been relatively poorly studied, especially in recent years. As a result, few data are available regarding the current distribution of hellbenders in the Susquehanna River drainage in Pennsylvania. Environmental DNA (eDNA) survey methods are becoming increasingly popular for some aquatic species and are highly conducive to obtaining rapid results for detecting populations of cryptic species. We re-examined the distribution of eastern hellbenders within the Susquehanna River drainage by testing for presence of hellbender eDNA primarily within streams that had historical population records. We assessed presence across 30 sites in 25 streams within the Susquehanna River drainage and the river itself during the 2015 breeding season (late August – September). We detected hellbender eDNA at 11 sites that included 9 streams and 2 locations within the Susquehanna River. Sites with confirmed hellbender populations were located in 6 of the original 18 counties in which hellbender populations were previously documented. Our results suggest that eastern hellbenders have undergone substantial range constriction within the Susquehanna River drainage in Pennsylvania.

Detection of the Eastern Hellbenders in the Susquehanna River Basin through Environmental DNA Technique

Mizuki Takahashi (Bucknell University), Carolyn McPhee (Bucknell University), Jordan R. Gaston (Allegheny College), and Matthew Venesky (Allegheny College)

Detection and monitoring of rare, elusive animals by sampling and analyzing the genetic material left in their habitats (environmental DNA or eDNA) is a noble conservation tool. The utility of eDNA sampling is not only cost- and time-effective but also non-disruptive to the animals and their habitats. Recent studies demonstrate that eDNA technique is effective in detecting eastern hellbender (*Cryptobranchus alleganiensis*) populations. The hellbender populations are rapidly declining throughout its entire distribution range. Pennsylvania and the Susquehanna River Basin harbored one of the best populations in the past. Yet, recent surveys revealed their declining status. In 2014, we conducted an eDNA survey of hellbender populations in 13 sites eight streams in the Susquehanna River Basin by sampling stream water monthly from June through October. Out of the eight streams surveyed, four are known to have hellbenders, serving as positive controls. The other four streams do not have official historical records of hellbenders. We also collected day and night water sample from each stream each month. By implementing this sampling regime, we aimed to test three hypotheses. First, eDNA of hellbenders would be detected from some of the streams that lack historical records. In particular, we were interested in one stream that has an anecdotal report. Second, hellbender eDNA concentration would increase during their breeding season in August and September. This trend was detected by the recent eDNA study conducted in North Carolina. Third, given their nocturnal nature, their eDNA concentration would become greater at night than during day time. We completed filtering of the collected water samples, DNA extraction, and quantitative PCR (qPCR) analyses. While we are currently finalizing data analyses, our preliminary analysis suggests that the eDNA concentration appears to increase during the breeding season and also at night.

Sequencing Characterization of Biotic Components Present in Potential Hellbender (*Cryptobranchus alleganiensis*) Habitats in Indiana

Ardith Wang (Purdue University), Obed Hernández-Gómez (Purdue University), and Rod N. Williams (Purdue University)

Next-generation sequencing (NGS) techniques are highly sensitive and can be used to characterize numerous micro- and macro- species that may have been previously unidentifiable. Hellbender salamander populations (*Cryptobranchus alleganiensis*) are currently declining and threatened range-wide possibly due to abiotic (habitat loss and pollution) and biotic (food scarcity, predation and disease) factors. Current hellbender conservation approaches include translocations of wild adults and re-introductions of captive reared individuals to areas where hellbender populations are low or extirpated. Biotic components of the habitat, such as predators and pathogen presence, may potentially negatively affect the survivorship of hellbenders released. We propose to use NGS to non-invasively identify possible hellbender inhabited streams and evaluate the biotic composition of promising reintroduction sites. We propose to perform 16s and 18s amplicon sequencing in order to characterize the bacterial and eukaryotic diversity within these environments. We predict hellbender eDNA will be detected in rivers currently known to be inhabited by hellbenders and that community diversity comparisons between rivers will differ in biotic components. Species diversity characterization should therefore aid in identifying suitable sites for hellbenders being reintroduced back into the wild.

Prevalence of a Chytrid pathogen (*Batrachochytrium dendrobatidis*) in Eastern Hellbender Salamanders in New York and Pennsylvania

Linxuan Wu (SUNY Buffalo State) and Amy M. McMillan (SUNY Buffalo State)

Amphibians are now facing the global declines. There are many possible causes to these declines, among which an emerging infectious disease, chytridiomycosis has drawn the attention of more and more scientists. Chytridiomycosis in the U.S. is mainly caused by the *Batrachochytrium dendrobatidis*. In this study, we detected the existence of this pathogen in the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) populations in the North Allegheny River Drainage of New York and Western Susquehanna River Drainage of Pennsylvania with the prevalence of 15%. According to our study, *Bd* has existed in the Allegheny River Drainage as early as 2004. Both swab samples and tail clips can be used for the *Bd* detection while swabs seemed to be more sensitive to the *Bd* detection.

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