



9TH HELLBENDER SYMPOSIUM

JUNE 17 – 20, 2019

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9th Hellbender Symposium logo art created by Mark Mandica,
Founder, Executive Director of The Amphibian Foundation, Atlanta GA

SPONSOR SPOTLIGHTS

Hellbender Brewing Company

Hellbender Brewing Company has been fermenting craft beer in Washington, DC since 2014. These guys were the first brewery on the east coast to incorporate a mash filter in to their brewing system. The mash filter increases efficiency by allowing them to brew faster while using 30% less water and 15% less grain each batch to achieve the same results as a traditional brewhouse – very cool!

Ben Evans, head brewer and CEO of Hellbender Brewing Company, comes from a career in microbiology and neuroscience research. He's been fascinated by hellbenders since youth and felt that promoting awareness for the species through their business name aligned perfectly with his ambition to incorporate sustainable and environmentally conscious techniques in the brewing process. Big thanks to Hellbender Brewing Company for sponsoring the canned beer selection served at Tuesday evening's poster session and social during the symposium!

<https://www.hellbenderbeer.com>



Independent Distilling Company

Independent Distilling Company was established in 2014 to produce small-batch handcrafted spirits that focus on local and quality ingredients in Georgia. Using a 100-gallon Copper Pot Still and a passion for spirits, co-founder Michael Anderson has honed his craft over the past years making traditional southern soulful spirits. The first product released was Hellbender Corn Whiskey made from locally sourced corn from Riverview Farms in Ranger, GA.

Much gratitude to the IDC crew for providing their Hellbender Corn Whiskey and Hellbender Bourbon Whiskey for a tasting offered at Tuesday evening's poster session and social!

<http://www.independentdistilling.com>



Mark Mandica, The Amphibian Foundation

Mark Mandica is the co-founder and executive director of The Amphibian Foundation – an Atlanta-based nonprofit that leads one-of-a-kind conservation programs to address threats to amphibians and their habitat in the southeastern United States and across the globe.

Mark is a life-long amphibian conservationist and leads novel conservation strategies focused on endangered species. He is also a biologist, scientific illustrator, musician, and most importantly, a father and a husband. Thank you to Mark for once again honoring the Hellbender Symposium by creating an awesome illustration for this 9th biennial event!

<https://www.amphibianfoundation.org/>



amphibianfoundation

Oskar Blues Brewery

In Fall 2018, The Pisgah Conservancy in North Carolina teamed up with Oskar Blues Brewery to craft a limited-release batch of Hellbender Helles. Sales from the special brew and feature events have benefited conservation initiatives of The Pisgah Conservancy that improve watershed habitat and outreach efforts for the hellbender.

Oskar Blues generously sponsored a portion of the 9th Hellbender Symposium branded steel pint cups that we hope will help us keep waste to a minimum at this event and for your future use – we appreciate their support and environmental stewardship!

<https://www.oskarblues.com>



KEYNOTE SPEAKER

Dr. Thomas K. Pauley

Professor Emeritus of Biological Sciences, Marshall University

Dr. Thomas Pauley has been studying amphibians and reptiles in the Central Appalachians since 1966, with a focus on salamander ecology in the West Virginia subalpine forest for over forty years. His major research species in these high elevations is the Cheat Mountain Salamander (*Plethodon nettingi*). Research highlights from his work with the Cheat Mountain Salamander include a study for the U.S. Forest Service on its range and distribution, a 31-year study of the effects of a ski slope on a population, a study on a transplanted population, numerous surveys for timber harvests and various types of rights-of-way, and several studies for the WVDNR, USFS, and USFWS.

Other studies have included the effects of diflubenzuron on forest terrestrial and stream salamanders, effects of Gypcheck and Bt on forest terrestrial and stream salamanders, effects of acid deposition on forest terrestrial and stream salamanders, potential effects of ultraviolet radiation on pond-dwelling amphibians, use of artificial road-rut pools by amphibians, and amphibian and reptile inventories in National Parks — New River Gorge National River, Bluestone Scenic River, Gauley National Recreational River, Harpers Ferry National Historic Park, and Capitol Region Parks.

In 1987, Dr. Pauley co-authored a book entitled “Amphibians and Reptiles in West Virginia”, published by the University of Pittsburgh Press. This is the only book published on amphibians and reptiles in the state. He is currently working on the second edition of this book and a companion volume entitled “An Atlas of Amphibians and Reptiles in West Virginia”. Additionally, he is working on a book about his experiences with amphibians and reptiles in the Central Appalachians, anticipated for publication in 2020.

Dr. Pauley was a professor of biology at Marshall University from 1987 to 2013, preceded by 21 years of college teaching at Salem College in West Virginia and the University of Pittsburgh at Bradford, PA. While at Marshall University, he was the major professor of over 90 graduate students whose research dealt with amphibians and reptiles of West Virginia, Pennsylvania, and Ohio. Dr. Pauley currently serves on several graduate student committees at Marshall University and West Virginia University.



AGENDA & PRESENTATIONS

JUNE 17 – JUNE 20, 2019

Monday, June 17

3:00 – 5:00 p.m. Registration & Check-In		Hyatt Place Hotel
5:00 – 5:45	Social/mixer Live music by Chloé and Stewart Scales	Holtzman Center Terrace
5:45 – 6:00	Opening remarks	Holtzman Center General Assembly Hall
6:00 – 7:00	Keynote Address: Fifty-two years searching hollows, ridges, and rivers in West Virginia Dr. Thomas Pauley, Professor Emeritus of Biological Sciences, Marshall University	
7:00 – 9:30	Dinner & Keynote Reception Live music by New Standard Bluegrass	Holtzman Center Terrace

Tuesday, June 18

6:30 – 9:00 a.m.	Complimentary breakfast for hotel guests	Hyatt Place Hotel
9:00 – 9:10	Welcome & Logistics	Hyatt Place Meeting Room
9:10 a.m. – 12:00 p.m. SESSION I: SURVEYS, MOVEMENT, AND STATUS ASSESSMENTS		
<i>Moderator: Paul Hime, University of Kansas</i>		
9:10 – 9:30	The Bumpy Road to Recovery of the Eastern Hellbender in Ohio Gregory Lipps, Ohio State University	
9:30 – 9:50	Ongoing Restoration of an Eastern Hellbender Population in the Upper Susquehanna River Watershed Michelle Herman (Grad), State University of New York	
9:50 – 10:10	Spatial ecology of Hellbender Salamanders prior to translocation Bradley Nissen (Grad), Tennessee State University	
10:10 – 10:30	Behavioral analysis of electroshocked post-metamorphic juvenile <i>Cryptobranchus alleganiensis bishopi</i> and subadult <i>Cryptobranchus alleganiensis alleganiensis</i> Stephanie Morrison (Grad), Missouri State University	
10:30 – 10:50	Break	

Moderator: William Hopkins, Virginia Tech

10:50 – 11:20 **A Species Status Assessment and 12-month petition finding for the Eastern Hellbender (*Cryptobranchus a. alleganiensis*)**

Jeromy Applegate, US Fish & Wildlife Service

11:20 – 12:00 Discussion: Species Status Assessment

12:00 – 1:20 Break for Lunch (on own)

1:20 – 2:40 p.m. SESSION II: JAPANESE GIANT SALAMANDER; PUBLIC ENGAGEMENT

Moderator: Mizuki Takahashi, Bucknell University

1:20 – 1:40 **Long term comparison in the estimated population and the structure of Japanese giant salamander in Matsuzai stream, Kita-Hiroshima-Cho, Hiroshima, Japan**

Yuki Taguchi, Asa Zoo, Hiroshima, Japan

1:40 – 2:00 **Effectiveness of the giant salamander bypass slopes at small dams in a small tributary stream, Tottori Prefecture, western Honshu, Japan**

Sumio Okada, Hanzaki Research Institute of Japan

2:00 – 2:20 **Engaging North Carolina's Trout Anglers and Other Stakeholders to Help Conserve Eastern Hellbenders**

Lori Williams, NC Wildlife Resources Commission

2:20 – 2:40 **Positive Perceptions About an Enigmatic Animal Follows the Conservation Biology Era: Content Analysis from 153 Years of Print and Online Media**

Shem Unger, Wingate University

2:40 – 3:00 Break

Moderator: Rod Williams, Purdue University

3:00 – 5:00 EXHIBITION SESSION: Education & Outreach Materials Hyatt Place Meeting Room & Foyer

5:30 – 6:00 Poster presentation set-up Lane Stadium South End Zone Club Room

6:00 – 9:30 Dinner & Poster Session Reception; State Updates Lane Stadium South End Zone Club Room
Music DJ Bobby Parker

Wednesday, June 19

6:30 – 9:00 a.m. Complimentary breakfast for hotel guests Hyatt Place Hotel

9:00 – 9:10 a.m. Welcome & Logistics Hyatt Place Meeting Room

9:10 – 11:50 a.m. SESSION III: DISEASE AND PHYSIOLOGY

Moderator: Amy McMillan, SUNY Buffalo State

- 9:10 – 9:30 **Associations between land cover and corticosterone profiles of free-ranging eastern and Ozark hellbenders from Missouri**
Cathy Bodinof Jachowski, Clemson University
- 9:30 – 9:50 **Neutrophil: lymphocyte ratios as a measure of chronic stress in populations of the hellbender (*Cryptobranchus alleganiensis*) across a habitat quality gradient**
Allison Litmer (GRAD), University of Arkansas
- 9:50 – 10:10 **Assessing Presence and Concentration of Herbicides and Heavy Metals at Current and Historical Hellbender (*Cryptobranchus alleganiensis*) Sites**
Jeronimo Silva, Tennessee State University
- 10:10 – 10:30 **Captivity-induced changes in the skin microbial communities of hellbenders (*Cryptobranchus alleganiensis*)**
Obed Hernandez-Gomez, University of California Berkeley
- 10:30 – 10:50 Break**
- 10:50 – 11:10 **Lesion Severity and Associated Skin Health Parameters in Endangered Ozark Hellbender**
Rebecca Hardman (GRAD), University of Tennessee
- 11:10 – 11:30 **Release of captive-raised Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) to test the success of a chytrid vaccine and cage release method**
Megan Kocher (GRAD), Buffalo State College
- 11:30 – 11:50 **Some Skin Secretions of Hellbender Salamanders: A Review**
Max Nickerson, University of Florida
- 11:50 – 1:20 Break for Lunch (on own)**
- 1:20 – 2:40 p.m. SESSION IV: ARTIFICIAL SHELTERS**

Moderator: Lorie Stroup, USDA Forest Service

- 1:20 – 1:40 **Use of Artificial Shelters (Nest Boxes) to Supplement Breeding Habitat for Hellbenders (*Cryptobranchus alleganiensis*)**
Jeff Briggler, Missouri Department of Conservation
- 1:40 – 2:00 **Resiliency of Artificial Shelters to Dynamic Stream Conditions in North Carolina**
Lauren Diaz (GRAD), Clemson University
- 2:00 – 2:20 **Improving the efficacy of artificial shelters as tools for studying eastern hellbenders: lessons learned over 5+ years of shelter deployment**
Sky Button (GRAD), Virginia Tech
- 2:20 – 2:40 **Detection of Hellbenders (*Cryptobranchus alleganiensis*) occupying artificial shelters using remote passive integrated transponder (PIT) tag scanning technology**
Brian Case (GRAD), Virginia Tech

2:40 – 3:00 Break

Moderator: William Hopkins, Virginia Tech

3:00 – 5:00 EXHIBITION SESSION: Artificial Shelters Hyatt Place Foyer

6:00 – 10:00 Dinner & Social; Best Graduate Student Platform Presentation Award Rising Silo Brewery
Live music by Clover Hollow

Thursday, June 20

6:30 – 9:00 a.m. Complimentary breakfast for hotel guests Hyatt Place Hotel

8:55 – 9:00 a.m. Welcome Hyatt Place Meeting Room

9:00 – 10:20 a.m. SESSION V: NON-INVASIVE MONITORING; CAPTIVE PROPAGATION

Moderator: Michael Freake, Lee University

9:00 – 9:20 **Development of an Instream Tag Monitoring System for the Eastern Hellbender Salamander**
Peter J. Petokas, Lycoming College

9:20 – 9:40 **Site occupancy and concentrations of hellbender environmental DNA in a large river**
Mizuki Takahashi, Bucknell University

9:40 – 10:00 **A Review of a Long-Term Program to Develop Reproduction Technologies for Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) at the Nashville Zoo**
Sherri Reinsch, Nashville Zoo

10:00 – 10:20 **Comparing the effects of environmental conditioning on survivorship of captive-reared hellbenders**
Nick Burgmeier, Purdue University

10:20 – 10:40 Break

10:40 – 12:00 Follow-up Discussions and Planning for 10th Hellbender Symposium

12:00 p.m. Adjourn

1:30 – 8:00 Social & picnic on the New River

LIVE MUSIC

Chloé and Stewart Scales

Hailing from opposite ends of the state of Virginia, Chloé and Stewart met in 2012 at the Floyd Country Store, brought together by a shared interest in the music of southwest Virginia. Chloé is a classically trained cellist who is exploring traditional music; Stewart is a self-taught bluegrass banjo, guitar, and bass player. Together they play a variety of styles, but folk and bluegrass are favorite genres. When they aren't playing music, Chloé teaches Latin and Special Education at William Byrd High School, and Stewart teaches Geography at Virginia Tech.

New Standard Bluegrass

New Standard was founded in October 2011 by Zach Brown, Spencer Blankenship and Mason Thomas. One month later, Stewart Scales was asked to be a part of the band as the bass player. Jason Wheeler was added on banjo in 2016. New Standard brings together a variety of styles and influences ranging from traditional artists to contemporary bluegrass and gospel artists. We believe in keeping traditional bluegrass music alive. New Standard plays a mix of traditional bluegrass and gospel music, honoring Bill Monroe, Flatt & Scruggs, Reno & Smiley and The Stanley Brothers (just to name a few). We also play original bluegrass and gospel tunes written by our band members. After 5 years of playing in western Virginia we hope to continue bringing music to the region for many years to come.

<http://www.newstandardbluegrass.com>

Bobby Parker

Bobby Parker is a Virginia Tech graduate and long-time Blacksburg resident. A former AM and FM radio DJ, at Virginia Tech's WUVT 90.7 college radio station and elsewhere, he's had a finger on the pulse of American popular music for decades. As a local musician and performer, solo singer/guitarist and with combos such as Attic Static, Hot Diggity Dog and Vinyl Tap, he understands the appeal of music for the body and the mind. He still owns several turntables and many, many records (and guitars!), and as a VT Forestry and Wildlife Management graduate ('79), he knows what the hell a hellbender is!

<https://www.facebook.com/bobbyparkermusic/>

Clover Hollow

Seated in southern Appalachia, Clover Hollow embodies the authentic roots music that surrounds them while producing a signature sound that is original, sharp and refreshing - blues-rock, bluegrass, old time, and old-school-country all meld together into the soul of their music. Inspired and skillful leads are offered up by Chris Printz (mandolin), Chris Burgoyne (guitar), and Joe Abercrombie (banjo and dobro), while John Munsell (string bass) holds up the groove. Laura Beth sings lead, plays rhythm guitar and writes the tunes that are now staples far beyond their hometown of Blacksburg, VA.

<https://www.reverbnation.com/cloverhollow>

ORAL PRESENTATION ABSTRACTS

Monday, June 17 – Keynote Address

Fifty-two years searching hollows, ridges, and rivers in West Virginia

Thomas Pauley¹

1. Marshall University, Huntington, WV

I have studied amphibians and reptiles in West Virginia since 1966 (52 years). In this presentation, I will share some observations I have witnessed and lessons I have learned. I have witnessed the discovery of new species, expansion of ranges, decline of species, and possible extirpation of species. Long-term searching revealed that some species that I thought had declined were present in good numbers when I searched during all seasons in appropriate habitats. During these 52 years, I spent 42 years concentrating on the distribution and natural history of the Cheat Mountain Salamander (*Plethodon nettingi*), as well as sympatric species. The Cheat Mountain Salamander is federally protected (threatened) and only occurs in high elevations of the Allegheny Mountains in West Virginia. While 52 years is a snapshot of time relative to the eons that amphibians and reptiles have been on earth, long-term data sets can provide us with population trends and potential causes of declines and extirpations.

Tuesday, June 18 – Session I: Surveys, Movement, and Status Assessments

The Bumpy Road to Recovery of the Eastern Hellbender in Ohio

Lipps, G.J.¹, Smeenk, N.A.¹

1. Ohio Biodiversity Conservation Partnership, Ohio State University

The Eastern Hellbender has been listed as a state endangered species in Ohio since 1990, following the completion of the first statewide survey in the mid-1980s. A follow-up survey in 2006-2009 found an 82% decline in relative abundance with most populations dominated by larger (older) individuals. These findings led to the formation of the Ohio Hellbender Partnership, made up of individuals from federal, state, and local agencies, as well as zoos, land trusts, and researchers, who came together to implement recovery activities. From 2011-2018, nearly 7,000 eggs have been collected from 28 nests in eight watersheds for captive head-starting, and nearly 1,000 individuals have been repatriated at 23 sites in eight watersheds. Artificial nest boxes (Hellbender Huts) are being placed throughout the state, to further investigate the causes of recruitment failure, aid in egg collection, and for monitoring populations. While Ohio's waterways have seen continual improvement since the passage of environmental laws of the 1970s, most of the state's waterways have less riparian forest cover and higher stream conductivity than what has been reported at sites with healthy populations. Further complicating recovery has been a recent dramatic increase in unconventional oil and gas activities in rural eastern Ohio, with fracing infrastructure degrading the habitat of the state's most robust populations. The road to recovery is a bumpy one for the Hellbender in Ohio.

Ongoing Restoration of an Eastern Hellbender Population in the Upper Susquehanna River Watershed

Herman, M.R.¹, Petokas, P.J.², and Gibbs J.P.¹

1. SUNY College of Environmental Science and Forestry, Syracuse, NY; 2. Lycoming College, Williamsport, PA

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) has been in decline since at least the mid-1990s in the Upper Susquehanna River drainage of New York, where stressors including flood events, industrial discharge, forestry and agricultural practices, and crayfish invasions have likely accelerated its disappearance from all but one known locality. To address this decline, we coupled site habitat enhancement with a headstarting initiative that investigated the influence of water source and diet during captivity on juvenile hellbender growth, health, and post-release outcome. Fertile eggs were collected in fall 2014 in the Pennsylvania and New York portions of the Susquehanna River watershed and reared by the Wildlife Conservation Society at the Bronx Zoo until age 2-1/2 years before transfer to a newly-constructed rearing lab in central New York. We assessed the condition of 99 juveniles at the lab on a monthly basis for one year. Prior to release in August 2018 the juveniles were PIT-tagged and we installed 200 pieces of sedimentary slab rock at the release site to serve as natural habitat. We also constructed and installed 20 artificial habitat structures and wire cages in the stream channel to serve as “soft release” locations. Following two weeks of acclimation inside the soft release environments, the juveniles were released to the wild and monitored using fixed and mobile PIT-tag reading systems. Our efforts to monitor the juvenile cohort were challenged by persistent and unseasonal high flows from mid-September 2018 through early 2019, but are ongoing.

Spatial ecology of Hellbender Salamanders prior to translocation

Nissen, B. D.¹, Nolan, E.¹, Freake, M.², Hardman, R.³, Sutton, W.¹

1. Dept of Agricultural and Envi. Sciences, Tennessee State University; 2. Dept of Natural Sciences and Mathematics, Lee University; 3. Center for Wildlife Health, University of Tennessee Knoxville

Successful translocation programs are dependent upon the quality of the habitats where animals are released, and a detailed knowledge of spatial ecology. Due to population declines throughout its range, the Eastern Hellbender salamander (*Cryptobranchus alleganiensis alleganiensis*) is a strong candidate for translocation in Tennessee. We used radio-telemetry to evaluate the spatial ecology (i.e. home range size and movements) and multi-scale habitat use of individual Eastern Hellbenders (N =27) in two sustainable populations to estimate suitable sites for translocations and to establish a baseline that can be used as a comparison after translocation. We recorded 377 location data points for 10 individuals in Tumbling Creek (~37 locations/individual) and 250 location data points for 17 individuals in the Hiwassee River (~ 14 locations/individual). Our preliminary results illustrate that most Hellbenders do not make frequent or large movements during summer months, and individuals spend most (86 -89%) of their time under large (>30 cm) boulders. The Hiwassee River Hellbenders had an average core home range of 30 m², a mean 95% KDE area of 300 m², and a mean MCP home range estimate of 355 m², whereas Tumbling Creek Hellbenders had an average core home range of 222 m², a mean 95% KDE area of 846 m², and a mean MCP home range estimate of 1584 m². We conclude that large boulders are a vital habitat requirement for translocation sites, and we predict that a successful translocation effort would involve hellbenders quickly locating suitable habitat and then displaying high site fidelity.

Behavioral analysis of electroshocked post-metamorphic juvenile *Cryptobranchus alleganiensis bishopi* and subadult *Cryptobranchus alleganiensis alleganiensis*

Morrison, S.M.¹, Briggler, J.T.², and Mathis, A.¹

1. Missouri State University; 2. Missouri Department of Conservation

Electrofishing, a common method of freshwater fish sampling, has been shown to negatively affect some fish species, but the effects on non-target species, such as hellbenders, have not been well studied. We tested responses of hellbenders to a range of voltage treatments. Post-metamorphic juvenile (~ 3 years old) Ozark hellbenders were tested in the laboratory, with individuals in higher voltage treatments having significantly greater incidences of secretions, twitching, and immobilization during exposure. Individuals in higher voltage groups also had lower righting scores and longer latencies to right. Subadult (~ 6 years old) eastern hellbenders were tested in both laboratory and natural river conditions. In laboratory trials individuals in higher voltage treatments had a significantly greater incidence of secretions, twitching, and immobilization during exposure, along with longer latencies to right. Field exposures occurred while hellbenders were under rocks in typical hellbender habitat in a river, and shocked subadults had higher incidences of skin secretions than control individuals. In a separate laboratory study, subadult hellbenders were exposed to two electrofishing events occurring three days apart. After both the first and second shocking events, hellbenders in higher voltage treatments had longer durations of twitching and immobilization during exposure. Individuals in higher voltage treatments also had shorter latencies to secrete and longer latencies to right. Two shocking events did not result in stronger responses than a single shocking event. Our results suggest that exposure to voltages similar to those experienced during typical electrofishing events resulted in at least short-term negative effects on hellbender behavior.

A Species Status Assessment and 12-month petition finding for the Eastern Hellbender (*Cryptobranchus a. alleganiensis*)

Applegate, J.A., S. Cameron, S. Chance, T. Crabill, B. Hosler, N. Rayman-Metcalf, and J. Szymanski

1. U.S. Fish and Wildlife Service

On April 4, 2019 the U.S. Fish and Wildlife Service published a 12-month finding in response to a petition to list the Eastern Hellbender (*Cryptobranchus a. alleganiensis*) as an endangered or threatened species under the Federal Endangered Species Act (ESA). Informed by a Species Status Assessment (SSA) completed in 2018, the agency determined that the species, overall, did not warrant listing but proposed that populations in Missouri be listed as an endangered Distinct Population Segment (DPS).

The SSA analyses indicated that although the total number of populations is expected to decrease over the next 25 years, numerous healthy populations are likely to persist across multiple adaptive capacity units. These populations will provide redundancy, resiliency, and representation at levels that are likely sufficient to sustain the subspecies now and into the future.

The Missouri populations of Eastern Hellbender are markedly separate from other populations, both genetically and by geographic separation, thereby meeting the definition of a DPS under the ESA. The lack of healthy populations and limited spatial extent of the DPS, coupled with the current and ongoing threats, puts the Eastern Hellbender in Missouri in danger of extinction.

Long term comparison in the estimated population and the structure of Japanese giant salamander in Matsuzai stream, Kita-Hiroshima-Cho, Hiroshima, Japan

Yuki Taguchi, Yoshinobu Minamigata, Noriyuki Nonoue, Hiroshi Kamada, Wataru Anzai, Moriyoshi Kubo, and Shinji Minami
Hiroshima City Asa Zoological Park

The Japanese giant salamander is listed on the red lists of the Japanese national government and many prefectures. They are an endangered species. However, their habitat information is very limited, for example there are a few distribution reports. To compare the data of population size and structure, we have conducted a 3-year (2014-2016) study of concentrated field surveys as we did in the 1990's (1994-1996). The study site was a 770 m stretch of Matsuzai stream in the Shijihara region, Kita-Hiroshima-Cho, Hiroshima, Japan. As a result, the estimated population was 37-78 individuals in this study and 41-120 individuals in the 1990's, which indicates a decline in this population. We calculated "Fulcon's Condition Factor (FCK)" to check body condition. FCK in the 1990's was 8.55 ± 0.26 (N=40). In contrast, in this study FCK was 7.30 ± 0.25 (N=26), indicating that individuals in the stream have become thinner (U-test, $p = 0.002$). This tendency was more substantial in individuals over 60 cm. The artificialization and fragmentation of the habitat could have caused a decline in food resources for the giant salamanders. We considered that it is more difficult for larger individuals to maintain bulkier body with a declining food supply.

Effectiveness of the giant salamander bypass slopes at small dams in a small tributary stream, Tottori Prefecture, western Honshu, Japan

Sumio Okada¹

1. Hanzaki (Japanese Giant Salamander) Research Institute of Japan

The Japanese giant salamander (*Andrias japonicus*) became a federally-protected species under the "special natural monument" designation in 1952, and is currently listed higher rank "vulnerable" from "near threaten" in the Japanese Ministry of Environment's Red Data Book, and is also listed "vulnerable" in the Tottori Prefecture's Red Data Book. Although catching this species is prohibited throughout its range, most of its habitat remains unprotected. Consequently, dams and concrete walls constructed for flood and erosion control, agriculture, hydraulic power generation, and road construction severely impact a large portion of *A. japonicus*'s riverine habitat, and dams are considered contributing habitat fragmentation of *A. japonicus*. In fact, many observations are reported that migrating *A. japonicus* are interrupted by dams during the breeding season. Recently, Tottori Prefectural government installed eleven bypass slopes at small dams (height: 20-160 cm) for conservation purpose of *A. japonicus* in a small tributary stream. I have been monitored these slopes using trail cameras and direct observations during breeding season between 2014-2018, and confirmed some individual salamanders got over the dams. I report effectiveness and issue of these slopes for conservation of *A. japonicus*.

Engaging North Carolina's Trout Anglers and Other Stakeholders to Help Conserve Eastern Hellbenders

Williams, L.A.¹, Rash, J.M.¹, Groves, J.D.², Stroup, L.L.³, and Blatny, D.⁴

1. NC Wildlife Resources Commission; 2. NC Zoological Park (Curator Emeritus); 3. U.S. Forest Service, Pisgah Ranger District; 4. NC Division of Parks and Recreation, New River State Park

Eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a protected species of concern in North Carolina. Despite long-term efforts by the North Carolina Wildlife Resources Commission (NCWRC) and partners to improve understanding of hellbender status in the state, census of all known and potential populations is lacking. The species' dependence upon clean, cold, well-oxygenated water restricts its distribution to North Carolina's Blue Ridge Ecoregion, overlapping much of the state's trout fishery. This overlap presented an opportunity for the NCWRC to educate anglers about hellbender conservation and supplement existing spatial and temporal species distribution data by enlisting angler help. In 2013, an advertisement within the North Carolina Inland Fishing, Hunting, and Trapping Regulations Digest initiated direct outreach on hellbender conservation needs to trout anglers. This advertisement complemented NCWRC's summer 2007-summer 2017 outreach efforts to a variety of stakeholders such as anglers, recreationists, and landowners that included posters, streamside signage, in-person programming and information tables, popular articles, and a nine-minute online documentary. These combined efforts resulted in 207 hellbender encounter reports from stakeholders, with 127 specifically from anglers. These data represent observations in 56 streams across 17 counties, with seven reports from waters that lacked previous knowledge of hellbender occurrence. Reports originated from private (n = 117) and public land (n = 90). The encounter method reported most often was incidental observation (n = 165). Much hellbender conservation work remains, but managers should consider exploring similar resource overlaps to collect valuable distribution data, help prioritize locations for monitoring efforts, and promote conservation messages.

Positive Perceptions About an Enigmatic Animal Follows the Conservation Biology Era: Content Analysis from 153 Years of Print and Online Media

Unger, S.D.¹, and Hickman, C.R.²

1. Biology Department, Wingate University; 2. Fisheries and Wildlife Management, Eastern Band of the Cherokee Indians

Print and online media may reflect changing perceptions about wildlife when viewed in a historical context and as conservation programs bring about increased awareness of declining species management. To determine whether public perceptions of a species with a proven history of public misunderstanding and persecution, the hellbender, change according to societal interests over time, we conducted a content analysis of 288 newspaper articles over the past 153 years of coverage through Conservation Era's, including: Exploitation (1850-1899), Protection (1900-1929), Game Management (1930-1965), Environmental Management (1966-1979), and Conservation Biology (1980-2016). In addition, we examined trends in more recent online media coverage. As measured by article frame (valence values), we detected an increase in positive perceptions about hellbenders in newspapers after 1980, which coincides with the Conservation Era. Among the most frequently-used words used to describe hellbenders pre-1980's, include "ugly" and "a monster" and "repulsive", whereas more recent words describing hellbenders include "unique" and "rare." Many articles before the Conservation Era included surprisingly informative natural history. Conservation efforts clearly had impacts on online media coverage,

which increased following the federal listing of Ozark hellbenders and their successful captive rearing by the St. Louis Zoo in 2011. These results are encouraging for conserving this enigmatic salamander.

Wednesday, June 19 – Session III: Disease and Physiology

Associations between land cover and corticosterone profiles of free-ranging eastern and Ozark hellbenders from Missouri

Bodinof Jachowski, C.M.¹, Briggler, J.T.², Millspaugh, R.J.³, and Millspaugh, J.J.⁴

1. Department of Forestry and Environmental Conservation, Clemson University; 2. Missouri Department of Conservation; 3. School of Natural Resources, University of Missouri; 4. W.A. Franke College of Forestry and Conservation, University of Montana

The physiological mechanisms behind hellbender declines remain largely unexplored. We investigated spatial variation in corticosterone profiles of free-ranging hellbenders from Missouri to better understand factors influencing hellbender physiology. Specifically, we investigated whether corticosterone profiles varied between subspecies, among genetically distinct population segments (i.e., rivers), as a result of local population density, land cover throughout the catchment and/or riparian areas, or with individual covariates. We collected a time series (baseline, 30 min, 60 min) of blood samples from 44 adults distributed across six rivers. We used mixed linear regression models to investigate factors associated with baseline and stress-induced corticosterone. All hellbenders exhibited a pronounced increase in corticosterone in response to handling (baseline: range = 0.21-18.28 ng/ml; 60 min.: range = 0.24-31.45 ng/ml). Corticosterone profiles were better explained by catchment land cover than by riparian land cover, river, subspecies, population density, or individual covariates. Baseline corticosterone concentrations were negatively correlated with catchment forest cover, suggesting that baseline energy demands of hellbenders increased as catchment forest cover declined. However, it remains unclear whether any hellbenders in our study suffered from chronic stress. Stress-induced corticosterone concentrations were also negatively correlated with catchment forest cover, suggesting that acute stress-responsiveness was not impeded in hellbenders with elevated baseline levels. Our findings suggest that land cover, or some correlate, has the potential to influence one aspect of physiological stress in hellbenders and emphasize the need for more work to investigate conservation implications of the patterns we describe.

Neutrophil: lymphocyte ratios as a measure of chronic stress in populations of the hellbender (*Cryptobranchus alleganiensis*) across a habitat quality gradient

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Amphibians are currently facing widespread population declines, primarily due to the introduction of anthropogenic stressors. Anthropogenic stressors have the potential to alter ecosystem dynamics and elicit long-term physiological responses that could hinder population viability. Current metrics of population viability rely on demographics, population fluctuation, and degree of isolation. Physiological parameters, such as stress-response proxies, offer a useful scale for population assessment, providing explanations underlying mechanisms causing population declines. Implicating a method of assessing chronic stress, such as white blood cell counts, specifically neutrophil: lymphocyte (N: L) ratios, may provide a metric for rapid assessment and relation back to

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microhabitat characteristics for management purposes. As a long-lived habitat specialist facing population declines, the hellbender (*Cryptobranchus alleganiensis*) is a good candidate species for applying N: L ratios to assess population viability. This study used N: L ratios in relation to microhabitat variables to assess chronic stress among populations, determine environmental variables correlated with stress, and assess the implication of N: L ratios among wild hellbender populations. Of the microhabitat variables assessed, high conductivity, low pH and low dissolved oxygen, correlated with elevated N: L ratios. Additionally, N: L ratios significantly differed among hellbender populations, suggesting that varying habitat condition and environmental stressors influencing population viability are reflected by N: L ratios. Specifically, N: L ratio indicated populations of management concern that traditional methods failed to suggest. Viability assessments incorporating physiological parameters provide evidence of declining viability earlier than traditional methods, and suggest specific variables contributing to stress, allowing for better management strategy.

Assessing Presence and Concentration of Herbicides and Heavy Metals at Current and Historical Hellbender (*Cryptobranchus alleganiensis*) Sites

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The Hellbender (*Cryptobranchus alleganiensis*) is a large, long-lived and fully-aquatic salamander species that is endemic to streams and rivers of the eastern United States. Hellbender populations have experienced drastic declines due to habitat degradation, accelerated sedimentation, aquatic contaminants, and infectious diseases. Although declining water quality is often suggested as a major contributing factor to hellbender population declines, few studies have evaluated the presence of aquatic contaminants at sites with current and historical *C. alleganiensis* populations. We assessed the presence and concentration of two commonly used herbicides (glyphosate and atrazine) and three heavy metals (Mercury [Hg], Cadmium [Cd], and Lead [Pb]) at thirty rivers across a watershed development gradient throughout the *C. alleganiensis* range in Tennessee and North Carolina. We developed a passive sampling technique that relies on osmosis to collect herbicides that are present in the water column or substrate. We believe this technique can be an effective tool to assess pollutant concentrations that *C. alleganiensis* individuals are potentially exposed to in contaminated sites. Our results indicated the presence of atrazine in 70% of our sites; six sites in Tennessee (min=0.068µM, max=2.654 µM) and 15 sites in North Carolina (min=0.001µM, max=0.092 µM). All sites contained cadmium (0.09 µg/g to 0.83 µg/g) and lead (1.22 µg/g to 10.4 µg/g), and 26 sites contained mercury (0.005 µg/g to 0.032 µg/g). Because this research focused on assessing the prevalence of commonly-encountered aquatic contaminants, study results are broadly applicable to hellbender populations and other aquatic biota throughout areas where these contaminants are found.

Captivity-induced changes in the skin microbial communities of hellbenders (*Cryptobranchus alleganiensis*)

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Captive environments often lack natural microbial reservoirs, have filtration systems that remove microbes, and are maintained in hygienic ways to prevent the spread of infectious disease. Aseptic conditions can negatively influence the establishment and maintenance of “wild-type” microbiotas within captive animals. Alternative microbial communities can result in the proliferation of disease among captive stock or upon reintroduction. Hellbenders (*Cryptobranchus alleganiensis*) are a threatened salamander for which extensive captive management is currently employed. Using metabarcoding, we characterized the skin microbiota of wild and captive-reared individuals from two subspecies in the state of Missouri, the eastern (*C. a. alleganiensis*) and the Ozark hellbender (*C. a. bishopi*). We also tracked changes in the skin microbiota of captive-reared eastern hellbenders exposed to river water from an intended re-introduction site in Indiana. Captive eastern hellbenders possessed richer communities than wild cohorts, whereas the opposite pattern was observed within the Ozark subspecies. We found significant microbial community structure between wild and captive populations of both subspecies. Eastern hellbenders exposed to river water in captivity had higher skin microbial diversity and distinct community composition on their skin, compared to control hellbenders. Exposing hellbenders to river water in captivity was an effective method to increase bacterial colonization and produced similar changes as release into the river. Our study provides a baseline for the effect of captivity on the skin microbial communities of hellbenders and highlights the need to incorporate microbiota management in current captive-rearing programs.

Lesion Severity and Associated Skin Health Parameters in Endangered Ozark Hellbender

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Arkansas populations of Ozark Hellbenders, *Cryptobranchus alleganiensis bishopi* are now reduced to a single river and over the past decade biologist have observed an increase of distal limb lesions with unknown etiology. We performed surveys of *C. a. bishopi* in Arkansas during summers of 2011-2014. We recorded biometrics, obtained a skin swab and tail punch biopsy, and recorded both qualitative and quantitative details of lesions present. In 2014 we collected an additional dorsal skin swab for microbiome analysis. We performed qPCR for presence of *Batrachochytrium dendrobatidis* (Bd) from skin swabs and Ranavirus from tissue. From lesion data we developed a scoring system from 0-7 and applied a score to each hellbender blind of associated information. We performed linear mixed model regressions followed by AICc model evaluation to determine effects of pathogen infection and individual biometrics on lesion score. We performed 16S amplicon sequencing from microbiome swabs and compared communities by lesion score. We discovered 93.2% of hellbenders had lesions characterized by digit swelling often progressing towards toe-tip ulceration. In severe cases we observed digital necrosis progressing to digit loss. Any recaptured individuals had the same or worse lesion score from previous captures. The top predictive model for lesion severity included individual mass and Bd infection status with a

significant, albeit weak, positive effect of Bd on lesion severity ($\beta=0.87$; C.I.: 0.11, 1.63). Microbiome results revealed decreased stability with severe lesion scores. Results demonstrate lesions are progressive, may be associated with overall skin health, and are likely multifactorial.

Release of captive-raised Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) to test the success of a chytrid vaccine and cage release method

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1. SUNY Buffalo State College

The reintroduction of captive-raised animals has been a fundamental response to the worldwide amphibian declines. Recent declines in the Eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) have resulted in several reintroduction studies which have had little success. Disease and movement away from suitable habitat have negative effects on the survival of released captive-raised hellbenders, further, there is evidence that chytridiomycosis (chytrid), a disease caused by the fungus *Batrachochytrium dendrobatidis* (Bd), may negatively impact hellbenders post-release. Information is still lacking regarding the progression of chytridiomycosis and its influence on post-release movement of hellbenders. This study tested a chytrid vaccine and cage release method to increase hellbender survival. Twenty captive-raised hellbenders were released into a stream in the Allegheny River drainage in June 2017. Half of these salamanders were vaccinated. Five vaccinated and five unvaccinated salamanders were released into cages until cages were removed in September. The other half (five vaccinated, five unvaccinated) were released directly into the stream. Hellbenders were located daily using radio telemetry and tested for Bd weekly for the remainder of the study period. Overall, the study resulted in just 25% survival. The vaccine was found to be unsuccessful with all hellbenders testing positive for Bd at some point during the summer. The results of this study also suggest that the caging method used is not effective at reducing hellbender movement. Further research is necessary in order to increase the success of captive-raised hellbender release programs.

Some Skin Secretions of Hellbender Salamanders: A Review

Nickerson, M. A.¹

1. University of Florida, Gainesville

Our lab has evidenced great variation in skin secretions in individuals and groups of hellbenders during capture, data taking, holding, and transport. Our review includes historic literature, written observations associated with our field data, and recent topical literature. This includes the white “mucous-like” material that we compressed into “balls with elastic characteristics”. Its’ presence seems tied with injury, dying, “fear of dying”, and abrasive events. We will discuss the evidence for this hypothesis.

Use of Artificial Shelters (Nest Boxes) to Supplement Breeding Habitat for Hellbenders (*Cryptobranchus alleganiensis*)

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1. Missouri Department of Conservation

Hellbenders have experienced severe population declines in Missouri with limited evidence of recruitment. With the multitude of threats for this aquatic salamander, as well as the decline, considerable resources have been devoted to propagation efforts to sustain and restore populations in Missouri. Part of these efforts is to collect eggs from the wild to hatch and head-start resulting larvae and juveniles at propagation facilities. Therefore, we designed and experimented with artificial shelters to increase our ability to obtain egg clutches. Three experimental prototypes were constructed and tested in the wild in 2008 and 2009 with some occupancy by adult hellbenders. Modifications of one of the original prototypes however, led to the first clutch of Ozark hellbender eggs deposited within a nest box in 2010. With this initial success achieved, additional nest boxes have been used throughout Missouri's rivers for both Ozark and eastern hellbender populations resulting in >60 egg clutches in the last 8 years. The use of such artificial shelters has also advanced the knowledge and understanding of breeding behavior and phenology of this species. This innovative approach has played a vital role in the recovery of this species in Missouri.

Resiliency of Artificial Shelters to Dynamic Stream Conditions in North Carolina

Lauren Diaz¹, Catherine M. B. Jachowski¹, Lori A. Williams², Shem D. Unger³

1. Dept. of Forestry and Environmental Conservation, Clemson University; 2. North Carolina Wildlife Resources Commission; 3. Carolina Headwaters, LLC

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a giant salamander inhabiting streams in the eastern United States that has experienced range-wide declines. Hellbenders are habitat specialists, and stream degradation from increased sedimentation is suspected as a leading cause of decline. Artificial shelters or "nest boxes" have recently been designed for hellbenders and may be useful for mitigating habitat loss from high sedimentation. Artificial shelters may be used to create breeding habitat where it has been lost as well as to monitor reproductive success and population stability. There are currently two designs with a variety of modifications in use throughout the hellbender range; however, no previous study has compared the designs for maximum efficiency. My objective is to compare three shelter types in terms of hellbender preference and probability that they will remain available to hellbenders. We installed 90 shelters among three stream reaches in Macon County, North Carolina, in the spring of 2018 and monitored them monthly from June-August. Due to no hellbender occupancy, we will move shelters to alternative streams in 2019 and continue to monitor them monthly from April-August 2019. This is an ongoing study, but our current data suggest that the probability of shelter availability depends on shelter type and site and ranges from 0.33 to 0.98.

Improving the efficacy of artificial shelters as tools for studying eastern hellbenders: lessons learned over 5+ years of shelter deployment

Button, S.T.¹, Jachowski, C.M.B.^{1,2}, Hallagan, J.J.^{1,3}, Case, B.F.¹, Groffen, J.¹, and Hopkins, W.A.¹

1. Dept. of Fish and Wildlife Conservation, Virginia Tech; 2. Dept. of Forestry and Environmental Conservation, Clemson University; 3. School of Natural Sciences and Mathematics, Stockton University

Artificial shelters show great promise as novel, non-invasive tools for studying hellbenders, but their use thus far has faced several challenges. During initial trials in multiple states, artificial shelters routinely became blocked by sediment and dislodged during high stream discharge events and were rarely used by hellbenders. We sought to determine whether these complications could be overcome via alternative shelter design, placement, and maintenance. Between 2013 and 2018, we deployed 438 artificial shelters of two different designs across ten stream reaches and three rivers in upper Tennessee River Basin. We tested several hypotheses, but postulated broadly that the availability, stability, and use of artificial shelters by hellbenders would depend on how shelters were constructed, deployed, and/or maintained. We found that factors previously limiting the feasibility of artificial shelters as tools for monitoring hellbenders could be mitigated under many circumstances. Specifically, we found that maintaining shelters at least once every 40 days limited sediment blockage, and building shelters with 3-4 cm thick walls, ~45 kg weight, and recessed lids improved their stability during high discharge events. Additionally, hellbenders most frequently occupied and nested in artificial shelters when they were deployed in deeper (~ 50+ cm) portions of reaches with high adult hellbender densities. Our results encouragingly suggest that artificial shelters can serve as effective tools for studying hellbenders when designed, deployed, and maintained with these advancements, but also highlight some limitations of their use.

Factors influencing in-situ detection of PIT-tagged Hellbenders occupying artificial shelters using a submersible antenna

Connock, J.R.¹, Case, B.F.¹, Button, S.T.¹, Groffen, J.¹, Galligan, T.M.¹, Hopkins, W.A.¹

1. Dept. of Fish and Wildlife Conservation, Virginia Tech

Traditional methods for studying hellbenders (*Cryptobranchus alleganiensis*) involves lifting rocks, which damages stream habitat, and is dangerous for both animal and surveyor. The use of artificial shelters has alleviated numerous risks posed to habitat, hellbenders, and surveyors during sampling. However, shelter arrays require tactile inspection to assess occupancy, and capture is necessary to identify occupants. We tested the potential for portable passive integrated transponder (PIT) scanning technology to remotely identify shelter occupants without the need for tactile inspection and occupant removal. We examined how the scanner's ability to detect tagged hellbenders occupying shelters was influenced by the presence and depth of cover rocks surrounding shelters, tag location within shelters, and tag orientation relative to the scanning equipment. We scanned 58 shelters with and without cover rocks, subsequently confirming scan accuracy (i.e., occupancy and tag identity) by inspecting shelters and capturing occupants. Of the 32 shelters occupied by a tagged individual, the scanner yielded a 31% detection efficiency with cover rocks in-place, versus 88% when cover rocks were removed. The scanner could not reliably (<50%) detect tagged individuals once cover rock depths exceeded 11cm. Tags located near the interface of the tunnel and chamber, as well as the chamber walls, had higher detection efficiencies than other locations of the shelter. Vertically oriented tags were 18% more likely to be detected than horizontally oriented tags. If further refined to maximize detection efficiency, this technology

could become a valuable tool for long-term population monitoring surveys, assessment of localized movement patterns, and shelter use.

Thursday, June 20 – Session V: Non-invasive Monitoring; Captive Propagation

Development of an Instream Tag Monitoring System for the Eastern Hellbender Salamander

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We designed, installed, and operated two instream passive integrated transponder (PIT) monitoring systems for the Eastern Hellbender during the summer through late fall of 2018. The systems were intended to monitor the activity of 99 three and one-half year-old juvenile hellbenders that were tagged and released in August 2018 to restore a historic, and largely extirpated, hellbender population in the Upper Susquehanna River watershed. Each monitoring system consisted of a hairpin-loop antenna that spanned a 20-meter wide stream channel and was anchored to the stream pavement with rebar and zip-ties. A tag reader was securely mounted on a streamside post and was connected to the antenna and to a control box located 30 meters landward. Each monitoring system received 24 volts of power from a bank of four solar storage batteries charged by a solar panel installed 30 meters distant in an open field. The control box contains a removeable flash drive that holds recorded tag data and an interface used to tune the system and to change system parameters. Since hellbenders are bottom crawlers, their tags are read when they walk across the antenna. The two monitoring systems provided data on movements of the juveniles into and out of the release sites, and on diel activity patterns. Most movements occurred late at night and, as such, would have been otherwise undetected.

Site occupancy and concentrations of hellbender environmental DNA in a large river

Hundermark, E.¹ and Takahashi, M.K.¹

1. Dept. of Biology, Bucknell University

Detection of threatened aquatic organisms using eDNA in a large river poses some challenges because of their microhabitat use, low eDNA concentrations due to their low abundance and stream current preventing eDNA diffusion laterally across the stream. The eastern hellbender (*Cryptobranchus a. alleganiensis*) in Susquehanna drainage serves as a good model species to examine sampling designs to overcome these issues. Their distribution is thought to be limited to some of the tributaries but not in the main stem. Our preliminary eDNA survey suggested the possible presence of hellbenders in the main stem. Given the preliminary data, the aim of the present study was to examine the importance of sampling season, vertical location along the stream stretch, and lateral location in detecting hellbender eDNA using occupancy modeling. In 2017, we collected water samples from four cross sections of the main stem (4 vertical locations), each of which contained five sampling sites spaced across the river width (20 sites) and water samples were collected repeatedly from those sites on monthly basis from July through October. We also collected water samples from three tributaries from July through October (92 total samples). We then analyzed presence/absence as well as concentration of eDNA in each sample. We repeatedly detected hellbender eDNA from multiple sites, corroborating the evidence of hellbender existence in the main stem. The best supported occupancy model contained “lateral location” as site

occupancy parameter and “breeding season” as detection probability parameter. Environmental DNA concentrations were also significantly higher during the September breeding season and in the left side of the river. The concentration estimates were significantly higher in the tributaries than in the main stem. Collectively, these results emphasize that it is important, when surveying a large river where eDNA concentrations are relatively low, to analyze water samples from both sides of the channel during the breeding season.

A Review of a Long-Term Program to Develop Reproduction Technologies for Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) at the Nashville Zoo

Reinsch, S¹, Trudeau, V.², Browne, R.³, and McGinnity, D.¹

1. Nashville Zoo; 2. Dept. of Biology, University of Ottawa; 3. Sustainability America, Sarteneja, Belize

An overview of a ten plus year project at the Nashville Zoo to develop Reproduction Technologies for eastern hellbenders in human care. Utilizing temperature and photoperiod cycling along with hormonal induction with timing based on ultrasound analysis, staff developed techniques to reliably collect eggs and milt from hellbenders maintained at the zoo. Through trial and error with In vitro Fertilization, new information was learned each year leading to the first eastern hellbenders being produced in human care. This work allowed staff and collaborators to develop cryopreservation techniques for hellbender milt and subsequently produce an offspring from cryopreserved milt. We continue to improve our techniques and hopefully can apply them other hellbender conservation projects in the future.

Comparing the effects of environmental conditioning on survivorship of captive-reared hellbenders

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1. Dept. of Forestry and Natural Resources, Purdue University; 2. United States Fish and Wildlife Service; 3. Indiana Department of Natural Resources, Division of Fish and Wildlife

Releasing captive-reared animals is a commonly used conservation tool to help improve declining wildlife populations. Captive-reared animals are often raised in conditions unlike their release sites which could further reduce survivorship post-release. We captive-reared juvenile Hellbenders for 18 months in standard aquaria with static water flow (control) and experimental raceways simulating flow conditions similar to their intended release sites (treatment). In November 2017 we released 80 radio-tagged individuals (40 treatment and 40 control) into soft-release structures in the Blue River. After release, we located each individual one to three times weekly through July 2018. Using a Cox-proportional hazards model, we found no effect of conditioning on post-release survival (Coef=0.3275; SE=0.3576; P=0.360), end location (Coef=0.2159; SE=0.3698; P=0.559), or weight (Coef=0.1993; SE=0.1843; P=0.280). Previous laboratory trials indicated that treatment animals showed no deleterious effects from the treatment and displayed improved swimming abilities compared to control animals. Further research will be conducted to determine if survival is affected by the timing of release and a combination of additional conditioning techniques.

POSTER ABSTRACTS

(Names in alphabetical order)

The Reproductive Ecology of the Recently Described Species of Leech (*Placobdella appalachiensis*) that Infects Eastern Hellbenders

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1. Dept of Fish and Wildlife Conservation, VA Tech; 2. Dept of Invertebrate Zoology, Smithsonian Institution; 3. Dept of Entomology, VA Tech

We recently identified a new species of leech, *Placobdella appalachiensis*, parasitizing eastern hellbenders in Virginia. In a combination of laboratory experiments and field work, we demonstrated that this leech transmits trypanosome parasites to hellbenders and influences their endocrinology and immunology. Therefore, it is important to understand the biology of these parasites to fully elucidate how they interact with their hosts. We sought to develop a comprehensive timeline of leech reproductive ecology and relate it to the timing of hellbender reproduction, provide a detailed description of leech offspring, and describe the reproductive behavior of adult leeches. Leeches were collected intermittently from May-September to determine the seasonal timing of their reproductive cycle. Leeches were also brought to the laboratory and observed daily. In addition, stored specimens collected in previous years were examined to further infer how leeches interact with hellbenders. All reproductive stages including spermatophore transfer, ovisac development, egg deposition, and hatchling attachment were observed and photographed. The average time (days) of each stage, measurements (length, width) of adults, eggs and hatchlings, and parental care behaviors (guarding and ventilation) were documented. Leeches appear to deposit their young on hellbender hosts for their first blood meal in mid-July to August, leading to increased leech prevalence and infection intensity coinciding with the onset of the hellbender reproductive season (late August). This work provides a foundation for understanding the seasonal dynamics of reproduction in a new species of ectoparasite in relation to its host's biology, which could contribute to conservation efforts for this declining amphibian.

Historical Relationship Between American Eel and Eastern Hellbender in the Upper Susquehanna Watershed

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An important and often misunderstood step in conservation is the successful restoration of a species into its native habitat. Little is known about the relationship of the Eastern Hellbender (*Cryptobranchus alleganiensis*) and the American Eel (*Anguilla rostrata*). Current restoration efforts are underway for both of these species in the Susquehanna River watershed. However, eel declines were underway before hellbenders had been well-studied in the region. Using a historical approach, we explored the ecological history of Eastern Hellbenders and American Eels. We used newspaper and scientific journal databases, as well as fishery records and state archives to determine the past distribution and abundance of both species in the Susquehanna watershed. Through the examination of these location-specific historical records, we assessed historic range overlap between these species. Preliminary data suggests that the American Eels and Eastern Hellbenders did overlap in distribution

throughout the lower reaches of the Susquehanna basin prior to the onset of eel decline. This case provides an excellent example of how historical ecology techniques may be applied to provide evidence and guidance for modern conservation management.

Investigating Habitat Selection Patterns of Larval Eastern Hellbenders in the Southern Appalachians

Lauren Diaz¹, Catherine M. B. Jachowski¹, Lori A. Williams², Shem D. Unger³

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The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a giant salamander inhabiting streams in the eastern United States that has experienced range-wide declines. It is estimated that hellbenders have declined by 70% in some portions of their range, and many populations are composed solely of older adults, suggesting a lack of successful breeding may be driving declines. Although successful reproduction and larval survival influences the long-term stability of hellbender populations, little is known about the ecological requirements of larvae. The eastern hellbender is currently under review for listing under the Endangered Species Act and understanding the requirements of their young is essential for accurately gauging population health and guiding conservation efforts. Our objective is to investigate associations between larval hellbender habitat use and abiotic factors hypothesized to influence survival. We selected streams in North Carolina with known hellbender populations to survey between May-August of 2018 and 2019, will survey for larvae using standardized cobbling methods, and will measure resource selection within a use/availability framework. This is an ongoing study, but we suspect larvae select habitat based on cover size and the stability of the microhabitat. This quantitative assessment of the likelihood larvae will select for a resource should guide the decisions of wildlife managers by providing objective criteria to evaluate habitat quality for hellbenders as well as to improve conditions that promote larval survivorship and gain a more robust understanding of the conservation status of hellbender populations.

Historical Insights on Conservation of the Eastern Hellbender (*Cryptobranchus alleganiensis*) in the Northeast

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1. Animal Behavior, Ecology and Conservation, Canisius College; 2. Biology Dept, SUNY Buffalo State; 3. Evolution, Ecology and Behavior Program, SUNY Buffalo

When planning for conservation and management of declining species, lack of historical data may pose challenges for those that have not been intensively studied or collected in the past. In the case of hellbenders (*Cryptobranchus alleganiensis*), few scientific surveys were conducted prior to the late 1970s and early 1980s, and official records of hellbender harvests were generally not kept. The resulting lack of information about historic distribution and relative abundance makes it difficult to determine the extent of hellbender declines or pinpoint when and why those declines began. This study uses a variety of sources, including newspaper archives, natural history expedition records, and personal accounts to map the historical distribution of hellbenders in New York, Pennsylvania, and Maryland. Information relating to hellbender mortality was also examined. Our results indicate that although hellbenders historically were fairly ubiquitous throughout the Ohio River basin, they had a restricted range with relatively low abundance in the Susquehanna River watershed. Early accounts

indicate that hellbenders may have been absent from the Susquehanna and its tributaries prior to the early 1800s, with reports from the West Branch Susquehanna increasing through the mid-1800s. This pattern may reflect a much later introduction to that drainage than previously believed. In addition, this research revealed a history of intense persecution of hellbenders by recreational stream users and commercial collectors that may help to explain some of the patterns of decline seen across this region today.

A proteomics approach to quantifying vitellogenin in the eastern hellbender (*Cryptobranchus alleganiensis*)

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1. Dept. of Biochemistry, Virginia Tech; 2. Dept. of Fish and Wildlife Conservation, Virginia Tech

The eastern hellbender (*Cryptobranchus alleganiensis*) is an imperiled aquatic salamander native to the eastern United States. Understanding the reproductive physiology of the eastern hellbender is vital to their conservation. Vitellogenin is a phospholipoglycoprotein synthesized in the livers of female vertebrates and delivered to developing ovarian follicles where it is cleaved into several yolk proteins essential for embryonic development. Since hellbenders are seasonal breeders, measuring seasonal variation in plasma vitellogenin concentrations can provide important insights into female reproductive cycles in the eastern hellbender. Vitellogenin has previously been detected in eastern hellbender plasma but the variation throughout the reproductive cycle has not yet been elucidated. In this study, we use an LCMS proteomics approach to quantify vitellogenin in female eastern hellbender plasma. We expect that circulating vitellogenin levels will gradually increase in the months leading up to oviposition and peak just prior to oviposition in early September.

Changes in circulating steroid hormone profiles before breeding in the eastern hellbender (*Cryptobranchus alleganiensis*)

Galligan, T.M.¹, Helm, R.F.², Case, B.F.¹, Bodinof Jachowski, C.M.³, Frazier, C.L.², and Hopkins, W.A.¹

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Seasonally breeding species such as hellbenders exhibit seasonal changes in circulating steroid hormone profiles that can provide critical insights into their reproductive physiology. We have determined the changes in circulating steroid hormone profiles throughout a four-month period preceding breeding in adult male and female eastern hellbenders using LC-MS/MS. This methodology provided the simultaneous determination of eight steroid hormones in hellbender plasma, including five androgens (testosterone [T], dihydrotestosterone [DHT], 11-ketotestosterone [11-KT], 11-ketoandrostenedione [11-KA], and dehydroepiandrosterone [DHEA]), two corticosteroids (cortisol [F] and corticosterone [B]), and one progestogen (progesterone [P4]). In both males and females, we observed a progressive increase in circulating T and DHT during the pre-breeding season, with both peaking in mid-August (17 days before breeding). 11-KT was only detected in females and also peaked in mid-August. These results suggest that T and DHT are the predominant androgens in eastern hellbenders, and are likely important regulators of reproductive processes in both males and females. The detection of significant quantities of DHT and 11-KT in females in August is particularly interesting, considering that, unlike T, neither of these hormones can be converted to estrogens. Therefore, some aspect of reproduction in the female eastern hellbender may be directly mediated by androgen signaling. Baseline F did not vary by season, but was higher in females. P4, 11-KA, DHEA, and B were rarely or never detected, and thus, do not appear to be important during

the pre-breeding season. This study provides an improved framework for future studies of hellbender reproductive biology.

Artificial shelters for juvenile and sub-adult hellbenders

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Artificial shelters have recently been deployed in several states as a possible method to study hellbenders. However, current shelters are rarely used by juveniles and sub-adults, making it difficult to determine their occurrence and abundance in declining hellbender populations, which are suspected to be suffering from a lack of recruitment. Thus, we tested a novel shelter design meant to target younger hellbenders. Our juvenile shelters have four separate chambers each with its own entrance and lid. To assess whether tunnel entrance size and/or orientation relative to stream flow influenced shelter use by hellbenders, each shelter contained two tunnel entrances of 3 and 5cm diameter, oriented in different directions. We installed 16 juvenile shelters in June 2018. Shelters were checked at approximately four-week intervals for five months to document occupancy, tunnel availability (blockage by sediment), and tunnel diameter preference. Shelters with accessible tunnels were occupied 19/78 checks, by 14 individuals. Occupying hellbenders had an average (\pm SE) total length of 35.9 ± 1.5 cm; 7.6cm shorter than in conventional artificial shelters. Five cm-wide tunnels were used more often (18/40) than 3cm-wide tunnels (1/38) when available. High stream discharge events of 120 and 1250cfs (2x and 23X annual median discharge) resulted in sediment blockages at 31% and 94% of the tunnels, respectively, which was unrelated to tunnel size or orientation. While juvenile shelters were successful at targeting younger hellbenders, their tendency to become blocked during high discharge events suggests that their design must be modified to improve utility.

Are Cryptic Species Really Cryptic? Morphometric Variation in *Cryptobranchus*

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Several recent studies have demonstrated significant genetic differentiation across the geographic range of the hellbender. These genetic findings have hinted at the possibility that there may be multiple "cryptic" species in the genus *Cryptobranchus*. But just how cryptic are these cryptic lineages? Using digital radiography and high-resolution CT scanning, we examine representatives from the four putatively most divergent lineages of hellbenders and assess morphometric variation across individuals and between lineages. Our results have bearing on the morphological distinctiveness of putative hellbender species and on the validity of potential species boundaries in this genus.

Chytrid and ranavirus prevalence in Eastern Hellbenders in Southeast Tennessee Streams

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Eastern Hellbenders are declining across historic ranges due to habitat loss, stream degradation, and disease. The chytrid fungus *Batrachochytrium dendrobatidis* is a major cause of amphibian species loss and is present in hellbender stream habitats. Ranaviruses are another group of pathogens that have been associated with mortality in Chinese Giant Salamanders, which are in the same family (Cryptobranchidae) as hellbenders. In this study, 27 hellbenders from two streams in southeast Tennessee were implanted with radio transmitters as part of a translocation project. Hellbenders were swabbed for chytrid once before transmitter implantation and opportunistically throughout the summer of 2018. Blood samples were also taken from individuals while under anesthesia for transmitter surgery. Overall, we will test samples for presence of *B. dendrobatidis*, *B. salamandrivorans*, and Ranavirus using qPCR. We will test for coinfection of the pathogens as well as prevalence differences between study site, size classes, sex, and overall seasonality. Preliminary results from 2018 showed that 63% of our study animals tested positive for Bd. Determining disease prevalence in these hellbenders is essential to monitor health of our study animals. It will also provide insight to amphibian disease susceptibility before and after translocation. All animals will be relocated in May 2019 to two different streams to augment declining populations, at which point they will again be sampled for chytrid and Ranavirus. We will continue to monitor for chytrid and Ranavirus in these populations after translocation in order to make informed conservation management decisions for this species.

Landowner Perspectives Regarding Science, Conservation, and the Eastern Hellbender

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The successful conservation of many species requires not only an in-depth knowledge of the animal, but also an understanding of how to successfully engage with private landowners who may have the species on their property. This is particularly true for species such as the eastern hellbender (*Cryptobranchus alleganiensis*) whose range is largely limited to privately owned land. Landowners have the potential to greatly assist in hellbender conservation by permitting research on their land, undertaking conservation activities, and/or supporting local conservation initiatives. However, they are unlikely to do so if the conservation activity is not aligned with their attitudes and beliefs. In this study, we sought to improve landowner engagement in hellbender conservation through a study of landowners in the Copper Creek watershed in Southwest Virginia. Using a mail survey (n=231), we measured landowner cognitions (e.g. attitudes and beliefs) regarding conservation and the eastern hellbender. Additionally, we assessed landowners' trust in science and the factors associated with landowner willingness to permit research on their property. We found that landowners who responded to the survey were generally trusting of science, supportive of conservation, and neutral towards hellbenders. The strongest predictors of how willing landowners were to permit research on their land included their 1) interest in learning about research, 2) attitudes about conservation, and 3) fear of new restrictions infringing on free use of their property. Such information can be used to tailor landowner engagement and encourage land management strategies that support hellbender conservation.

Estimating allelic diversity through hellbender eDNA sampling

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Environmental DNA monitoring holds great promise for population assessment of secretive species such as hellbenders. While eDNA has contributed significantly to presence/absence surveys, the success of the method for assessing population status is much more equivocal. Estimating allelic diversity through eDNA collection is a potential approach for approximating population size and viability. Typical eDNA markers are based on mitochondrial genome regions, which lack extensive variation within populations. Some nuclear genetic markers, such as microsatellites, are more variable, but more difficult to successfully amplify in environmental samples due to fewer copies relative to mitochondrial. We tested a nested PCR approach for amplifying nuclear microsatellites using water collected from The Wilds Hellbender Conservation Center. We collected up to 5 liters of water from three tanks and swabbed all the hellbenders in the tank to collect individual genotypes. Our three tanks included: 1) a single 5-year-old hellbender in a 30-gallon tank; 2) two adult hellbenders in a 300-gallon tank; and 3) five 4 year old hellbenders in a 40 gallon tank. We were able to successfully amplify microsatellite DNA from all three tanks and identify all known alleles across multiple replicates. The eDNA samples had extensive allelic dropout, which is often seen in low-copy DNA samples, and therefore many PCR replicates will likely be required, especially in field situations. Given the high variability in hellbender microsatellites, and up to 12 loci available, we anticipate there may be potential to estimate population size through this method.

Detection of Japanese giant salamanders (*Andrias japonicus*) in first-order streams using environmental DNA

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The populations of the Japanese giant salamander (*Andrias japonicus*) have experienced declines over the past several decades, which led to the recent change in its conservation status from "Near Threatened" to "Vulnerable" by the Ministry of Environment. One of the major conservation issues is concrete banking. Concrete banking not only deprives them of nesting cavities along stream banks but also can cause habitat fragmentations between a main stem and its small tributaries. Although these small tributaries are often too small for adult salamanders to utilize, it has been speculated that larval and juvenile salamanders may inhabit these tributaries to avoid competition and predation. In summer 2018, we surveyed three small first-order tributaries of Ichikawa River in Hyogo Prefecture. Starting from the congruence with the main stem, we walked up each tributary and collected water samples every 20 to 50 meters until the streams completely died (Tributary A: 465m long, N=8; Tributary B: 955m long, N=21) or became less than 50cm wide (Tributary C: 2331m long, N=22). We filtered those samples, extracted DNA from the filters, and ran quantitative PCR (qPCR) on each sample. Although no giant salamanders were observed during the field sampling, we repeatedly detected giant salamander eDNA in all tributaries. These results suggest that small tributaries likely provide important habitats for larval and juvenile giant salamanders that most likely hide underneath rocks and gravels. Maintaining habitat connectivity between a main stem and its small tributaries should be considered as an important part of the conservation plan.