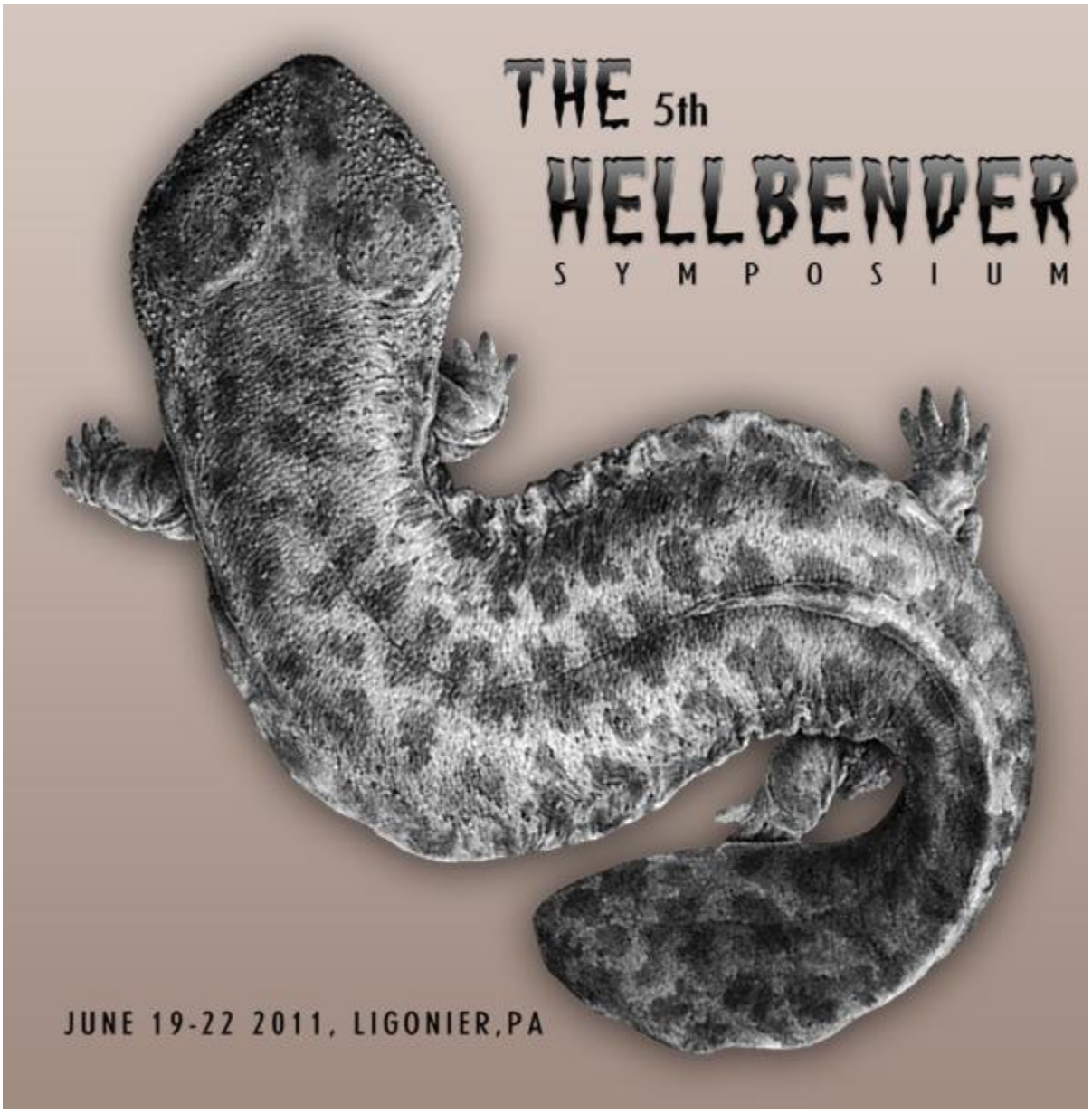

Antiochian Village Conference Center

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5th Hellbender Symposium
19-22 June 2011, Antiochian Village, Ligonier, Pennsylvania

Sunday, June 19

4:00 pm	Check in at Antiochian Village Conference and Retreat Center	
7:30	Welcoming remarks and field trip details	Coffee House
8:00 – 11:00	Social/Mixer	Coffee House

Monday, June 20

8:00 am	Continental Breakfast	Dining Hall
8:30-9:00	Depart for field trips to local rivers	
5:30 pm	Return to Antiochian Village	
6:00	Dinner	Dining Hall
7:30	***Keynote Address, Al Breisch, NY DEC (retired)***	
	I Have Met the Hellbender	Ajar Ampitheater
9:00 – 11:00	Social/Mixer	

Tuesday, June 21

8:00 am	Breakfast	Dining Hall
9:00	Opening remarks	Ajar Ampitheater
9:15-12:15	**Session I: Surveys and Status Assessments**	Ajar Ampitheater
9:15-9:35	*Documented Decline of the Eastern Hellbender in Loyalsock Creek in North-Central Pennsylvania* Peter J. Petokas	
9:35-9:55	*A Historical Assessment of the Eastern Hellbender* (Cryptobranchus a. alleganiensis) *in a Pennsylvania Stream* Matthew Kaunert	
9:55-10:15	*Observations of Hellbenders in Two Different Southwestern Pennsylvania Watersheds: Implications for future management and conservation* Eric J. Chapman and Rebecca Allshouse	
10:15-10:35	*Surveillance of the Eastern Hellbender in West Virginia* Joe Greathouse	
10:35-10:50	**Coffee/Tea Break**	Upper Museum Lobby
10:50-11:10	*Eastern Hellbender Survey Results and Conservation Efforts in North Carolina* Lori A. Williams and John D. Groves	
11:10-11:30	*Update on the Ozark Hellbender: Eleven Point River, Arkansas* Kelly J. Irwin	

11:30-11:50	*Range-wide hellbender status mapping: An update* Jeff Humphries and many others	
11:50-12:15	Around the room State by State status assessment	
12:30-1:30 pm	**Lunch**	Dining Hall
1:30-2:50	**Session II: Health, Disease, Education**	Ajar Ampitheater
1:30-1:50	*Health and habitat quality assessment for hellbenders in Indiana* Zachary H. Olson, Nicholas G. Burgmeier, Shem D. Unger, and Rod N. Williams	
1:50-2:10	*Ectoparasitism, Innate Immunity, and Stress Physiology of Eastern Hellbenders (*Cryptobranchus alleganiensis*) from Stream Reaches with Differing Habitat Quality* W.A. Hopkins, S.E. DuRant, W.E. Moser	
2:10-2:30	*Beyond Chytrids* C. Nickerson, C. M. Ott, S.L. Castro, V. M. Garcia, T. C. Molina, J. T. Briggler, A. L. Pitt, J. J. Tavano, J.K. Byram, M.A. Nickerson	
2:30-2:50	*Education of Local People About* Andrias japonicus *and an Introduction to the Japanese Giant Salamander Society.* Sumio Okada	
2:50-3:10	**Coffee/Tea Break**	Upper Museum Lobby
3:10-4:50	**Session III: Recovery, Husbandry, and Headstarting**	
3:10-3:30	*An update on the Ron Goellner Center for Hellbender Conservation* Chawna Schuette	
3:30-3:50	*Conservation and Recovery Plan for the Eastern Hellbender in Ohio* Gregory Lipps	
3:50-4:10	*Survival and Body Condition of Captive-Reared Juvenile Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) Following Translocation to the Wild* Catherine M. Bodinof, Jeffrey T. Briggler, Randall E. Junge, Tony Mong, Jeff Beringer, Mark D. Wanner, Chawna D. Schuette, Jeff Ettling, Joshua J. Millspaugh	
4:10-4:30	*Hellbender sperm on ice* McGinnity D, Nofs S, Kirk M, Agnews D, Carlton C, Figiel C., Browne RK, Vercammen F., Uteshev VK, Shishova NR, Gakhova EN, Mansour N., Wu M, Li H, Schaftenaar W., Good Zoo, et al.	
4:30-4:50	*Conservation of Hellbenders (*Cryptobranchus alleganiensis*) in the Ozark Highlands* Jeffrey T. Briggler	
6:00	**Dinner**	Dining Hall
8:00	Video Night	TBA

Wednesday, June 22

8:00 am	Breakfast	Dining Hall
9:00 – 10:40	**Session IV: Ecology and Behavior**	Ajar Ampitheater
9:00-9:20	*Ecology of larval hellbenders in a Great Smoky Mountains stream* Kirsten Hecht-Kardasz, Michael Freake, Max A. Nickerson, and Phil Colclough	
9:20-9:40	*Seasonal movements of the Japanese giant salamander (*Andrias japonicus*)— Evidence for possible breeding migration by this stream-dwelling amphibian.* Yuki Taguchi	
9:40-10:00	*Field Observations of Parental Care of Egg Masses by Male Japanese Giant Salamanders,* Andrias japonicus Sumio Okada, Yukihiro Fukuda, Etsuko Yuki, and Tamami Okada	
10:00-10:20	*Abundance and Spatial Ecology of Eastern Hellbenders in Indiana* Nick Burgmeier, Shem Unger, Trent Sutton, and Rod Williams	
10:20-10:40	*Natural History Notes on the Eastern Hellbender in West Virginia* Joe Greathouse	
10:40-11:00	**Coffee/Tea Break**	Upper Museum Lobby
11:00-12:00	**Session V: Genetics**	Ajar Ampitheater
11:00-11:20	*The Search for Sex-Linked Genes in Cryptobranchid Salamanders: Genome-Wide Gene Discovery and Marker Development* Paul Hime, David Weisrock, Justin Kratovil, and Joshua Reece	
11:20-11:40	*Population Genetics of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) at multiple spatial scales* Shem D. Unger and Rod N. Williams	
11:40-12:00	*Historical and Contemporary Patterns of Genetic Connectivity among Hellbender Populations across the Tennessee Valley* Stephen Spear, Michael Freake, Eric Routman, Rebecca Johnson, and Christopher Jenkins	
12:00 pm	Wrap-up and plan for 6th Hellbender Symposium	
1:00	Adjourn	

ABSTRACTS

ORAL PRESENTATIONS

Monday, June 20: Keynote address

I have met the Hellbender

Alvin R. Breisch, New York State Department of Environmental Conservation (retired)

The amphibian and reptile species found in the Northeast today are the result of a series of invasions that followed the retreat of the Laurentide Ice Sheet about 11,000 years ago. Although Native Americans utilized these species in a variety of ways, little of this use is documented in early literature or archaeological digs. European settlement of the Northeast has resulted in significant changes to the native populations of amphibians and reptiles, some through direct action but much through the unintentional consequences of other actions. Amphibians and reptiles were originally exploited or persecuted by the early settlers. Recognition of herp diversity peaked during the "Naturalist Period" which extended from the early 1700s to the late 1800s. This transitioned into the modern era around the beginning of the 20th century when popularizing, scientifically studying and protecting amphibians and reptiles became the standard. Although still threats today, exploiting and persecuting herps have to a large degree been replaced by acceptance, appreciating and understanding that they are an important part of our natural heritage. Since its discovery in 1801 the fate of the hellbender has mirrored these changes in threats and attitudes. James DeKay (1842) stated in the "Zoology of New York" that "I have never met this animal myself." I am delighted to say that "I have met this animal."

Tuesday, June 21- Session I: Surveys and Status Assessment

Documented Decline of the Eastern Hellbender in Loyalsock Creek in North-Central Pennsylvania

Peter J. Petokas

Surveys conducted in Loyalsock Creek, a tributary of the West Branch of the Susquehanna River, from 2006-2010 have shown a dramatic decline in the Eastern Hellbender population. Hellbender morality is severe and has been frequently reported by sportsmen, kayakers, swimmers, and SCUBA divers, with some reports from as far back as the mid-1990's. Although hellbenders still occur in the creek, they are few in number and population recovery seems improbable. Only a few of the recovered carcasses had been pit-tagged as part of an ongoing mark-and-recapture study. Possible causes of the mortality include parasites, disease, and chemical pollutants such as pesticides and endocrine disruptors. This decline parallels similar declines in hellbender populations in tributary streams of the Main Stem and North Branch of the Susquehanna River over the past two decades.

A Historical Assessment of the Eastern Hellbender (Cryptobranchus a. alleganiensis) *in a Pennsylvania Stream*

Matthew Kaunert

A historical assessment of the Eastern Hellbender *(Cryptobranchus a. alleganiensis)* was undertaken in the summer of 2010 in French Creek, a tributary of the Allegheny River in northwestern Pennsylvania. The primary purpose was to re-sample the historic population sampled in 1968 by Hillis and Bellis (1971) and to compare population size and structure in an effort to provide

comparative data on the status of the species in Pennsylvania. A second goal was to sample four other sites to obtain a broader survey of the status of the species in the middle reaches of the French Creek drainage system. Two of these sites were located above Meadville and two below. I found a 50% decline in the density of salamanders at the Hillis and Bellis (1971) site; however, all size classes found in the original study were again found at the Hillis and Bellis (1971) site as well as one other nearby site (McGuffintown). The presence of subadults and gilled larvae at the historic site indicates there has been recent reproduction and recruitment in the population. The population size of hellbenders within sample plots at the four other sites varied considerably, with the total number of animals captured above Meadville (130) outnumbering those below (40). The populations above Meadville also included a larger range of sizes, including juveniles and larvae, whereas those below did not. These data suggest a longitudinal trend favoring large, sexually mature adults farther down the longitudinal gradient of the stream. Further conservation efforts and an expanded coverage of sampling efforts are needed to better understand and protect this declining salamander species.

Observations of Hellbenders in Two Different Southwestern Pennsylvania Watersheds: Implications for future management and conservation

Eric J. Chapman and Rebecca Allshouse

Current Eastern Hellbender (*Cryptobranchus alleganiensis*) populations in many western Pennsylvania streams are largely unknown. Western Pennsylvania Conservancy (WPC) in cooperation with the Pittsburgh Zoo and Pittsburgh Plate and Glass (PPG) Aquarium, and Clarion University staff have been surveying for Hellbenders in two watersheds where historic populations had been documented. Eastern Hellbenders were located in Little Mahoning Creek, Indiana County, and Tubmill Creek, Westmoreland County during surveys conducted in summers of 2007 through 2010. Some previously tagged animals by the Zoo staff in 2005 have also been recovered. We conducted rock-turning surveys, with a total of 1,768 person hours spent between the two watersheds searching for all life history stages of Eastern Hellbenders. A total of 137 individual Hellbenders were tagged between 2007-2010 field seasons. Of those, 16 animals are repeat captures from 2007, 15 are recaptures from 2008, and 13 are recaptures from 2009. In addition, 9 individuals were recaptured from 15 tagged by the Pittsburgh Zoo and PPG Aquarium surveys in 2005 and 2006. Observations on meristic changes, individual home ranges, CPUE estimates, and physical abnormalities were recorded for all animals. The majority of individuals captured ranged in size from 40-60 cm. The under representation of smaller size classes could be a product of using only rock turning as our capture method and a skewed population towards older animal indicating possible low recruitment rates in both watersheds.

Surveillance of the Eastern Hellbender in West Virginia

Joe Greathouse

During 2010, abundance estimates of Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) were calculated from two rapid assessments of hellbender populations at sites on 23 of 27 creeks and rivers that were sites of historic occurrence of hellbenders in West Virginia. Hellbenders were documented at 12 of these 23 sites. In addition to these capture per unit effort measurements, in-stream habitat data associated with substrate composition, prey density, and water chemistry were collected. Threats to populations of this species in West Virginia have been speculated to include acid mine drainage and siltation, but abundance estimates for this species in West Virginia and their associations with habitat variables have not been quantified.

Eastern Hellbender Survey Results and Conservation Efforts in North Carolina

Lori A. Williams (NC Wildlife Resources Commission) and John D. Groves (NC Zoo)

In North Carolina, the Eastern Hellbender (*Cryptobranchus a. alleganiensis*) is state-listed as a Special Concern, priority species. Previous status assessments have regarded North Carolina's hellbender populations as "stable." However, we lack the historical population data needed to make that determination. In 2007 the NC Wildlife Resources Commission, the NC Zoo, and many other partners began baseline inventories of historically known sites as well as new, potential sites in all five NC mountain river basins where hellbenders occur. We have successfully updated hellbender records in 77% of all historical sites surveyed and documented hellbender presence at more than 20 new sites. In most surveys (less than 15%), we are not finding larvae or very young juveniles, which could potentially indicate low reproductive success. We are also conducting disease surveillance on captured hellbenders, documenting chytrid fungus in all five hellbender river basins in North Carolina (22% of samples positive) and ranavirus in one river (0.02% of samples positive). However, we are witnessing no physical signs of acute infection or illness in captured animals. Other topics we will discuss include habitat data collection, education/outreach efforts, partnerships, and future directions for hellbender conservation in North Carolina.

Update on the Ozark Hellbender: Eleven Point River, Arkansas

Kelly J. Irwin

In 2005, a long-term population monitoring program was initiated on the sole remaining Ozark Hellbender population in the Eleven Point River in Randolph County, Arkansas. The well known and historically robust Spring River population is now considered functionally extinct and the only extant population in the state occurs in the Eleven Point River. Data from this project builds upon that of B. Wheeler (2001-2004 SWG funded work). Some 219 individuals have been PIT tagged along the ~32 km reach. SVL frequency is normally distributed and mean sex ratio is 2.6:1 (male:female). Almost half (n=22) of the 51 recaptures are 4+ years in duration (range 1-8 yrs). Mean CPUE is 1.0/hr and mean capture rate is 41.3 individuals per biennial survey. Three long-distance movements have been recorded; two were significant (~770 m upstream and ~1000 m downstream). Persistent problems are habitat degradation (sedimentation and livestock access) and disease/ health issues (Bd positive tests and digit/ foot loss). Although empirical proof is lacking at present, it is suspected that loss of digits and feet is related to the presence of Bd which allows flesh eating bacterial growth on the feet. In spite of these negative impacts on the population, three juveniles were located in the 2009 survey which gives us hope that this population will persist until conservation efforts can address these problems.

Range-wide hellbender status mapping: An update

Jeff Humphries and many others

An assessment of the range-wide status of the hellbender, *Cryptobranchus alleganiensis*, is still being developed, based on field survey data from hellbender researchers. Numerous researchers have contributed additional data since this project was first presented at the 2009 Hellbender Symposium, and we are now nearing completion of a publication outlining this work. Information about presence-absence, relative abundance, and the presence of juveniles (recent reproduction) is being compiled into an index of hellbender conservation status at the HUC-8 watershed level throughout the range of the species. This information should guide the Federal listing process, as well as provide information about where additional survey effort and watershed protection should occur.

Tuesday, June 21 - Session II: Health, Disease, and Education

Health and habitat quality assessment for hellbenders in Indiana

Zachary H. Olson, Nicholas G. Burgmeier, Shem D. Unger, and Rod N. Williams

There are many factors purported to negatively impact hellbender populations, but little empirical data exist to diagnose the causes of declines. We assessed blood chemistries, physical abnormalities, the presence of Chytrid, and other environmental variables to determine if declines in Indiana could be attributed to the overall health of the hellbenders. We analyzed blood collected from 86 individuals during 2008-2009 for hematological parameters and the presence of blood parasites. Most parameters were similar between the sexes, and there was no evidence of hemoparasites in the population. Sperm quality was high among the 8 males we assessed. Physical abnormalities occurred frequently (60 of 80; 68.2%) although the majority were due to physical traumas rather than ontogenetic causes. We detected amphibian chytrid fungus in one adult male out of 77 individuals assessed. Finally, analysis of 264 water samples collected from November 2008 to August 2009 indicated that the Blue River exhibited adequate water quality for hellbenders. Three pesticides were detected over the course of sampling, and atrazine was detected at levels that have been problematic for other amphibians. However, we found no detectable effects concerning the endpoints of VTG production and sperm quality in the Blue River population. In fact, VTG assays were over 98% effective as a sex determining biomarker up to 2-months prior to the breeding season. We are conducting additional trials using repeatedly-sampled females to determine which months VTG is a reliable indicator of sex.

*Ectoparasitism, Innate Immunity, and Stress Physiology of Eastern Hellbenders (*Cryptobranchus alleganiensis*) from Stream Reaches with Differing Habitat Quality*

W.A. Hopkins, S.E. DuRant, W.E. Moser

Much like other vertebrates, habitat loss and degradation is perhaps the greatest direct threat to many amphibian populations around the world. In addition to depriving amphibians of physical habitat requirements (e.g., shelter), habitat modification may also effect the health of amphibians and potentially precipitate interactions with other deleterious factors such as pathogens, contaminants, and invasive species. The current study was designed to provide background information about the physiological state of eastern hellbenders (*Cryptobranchus alleganiensis*) experiencing different surrounding land use that influences in-stream habitat quality. Hellbenders are one of the largest amphibians in the world and have recently experienced dramatic population declines. Habitat degradation and disease appear to underlie some hellbender population declines. When comparing hellbenders from a stream reach with greater anthropogenic disturbance to a more forested site, we found baseline and stress-induced plasma levels of corticosterone, as well as innate immune responsiveness (i.e., blood killing ability), were similar in the two areas. Males consistently had higher plasma corticosterone levels than females, a finding consistent with the territorial activities of males early in the breeding season. Moreover, we discovered a high incidence of parasitism by leeches in the more agriculturally-impacted site. Morphological analyses confirm that the leech is the same species commonly found in declining Ozark hellbender populations in Missouri, but this parasite has never been documented in eastern hellbenders. We discuss several hypotheses as to why this parasite is found in our study system and the potential health implications for hellbenders.

Beyond Chytrids

C. Nickerson, C. M. Ott, S.L. Castro, V. M. Garcia, T. C. Molina, J. T. Briggler, A. L. Pitt, J. J. Tavano, J.K. Byram,* M.A. Nickerson * = Presenting & corresponding author

About 50% of the Ozark Hellbenders *C. a. bishopi* within the original NFWR research section of Nickerson and Mays (1973a, b) have substantial injuries. We captured six adults on 17 August 2007, all with injuries. Each individual was placed into a clean bucket filled with river water, measured, weighed, visually inspected for presence of leeches, injuries, or abnormalities, and photographed. The feet/limbs showing signs of infection (e.g., lesions, sores or exposed bone) were swabbed with sterile, buffered swabs and the lower lip of one individual, with a raw sore was swabbed. Swabs were streaked onto three different microbiological culture media: Sabouraud Dextrose Agar, Mannitol Salt Agar, and sheep's blood agar to obtain a broad diversity of microbes. Two feet with no signs of infection from two *C. a. bishopi* served as controls. Sample plates were secured in ice-packed styrofoam coolers and flown to the Microbiology Laboratory at NASA Johnson Space Center for microbial identification. Bacterial isolates were identified with the Vitek 2 system biochemical analysis (bioMérieux). Bacterial isolates not identified by this system were identified by 16S ribosomal DNA sequencing using a MicroSeq 500 16S rDNA Bacterial Identification Kit (Applied Biosystems). Fungal isolates were identified by microscopic morphological characteristics.

While bacterial and fungal isolates were generally characterized as common environmental organisms, several opportunistic pathogens, including those known to form biofilms, were identified associated with only injured tissues of *C.a. bishopi.* While no common organism was isolated from all wounds, the repressed immune system of the injured animals may have increased their susceptibility to opportunistic pathogens, potentially contributing to, either alone or in combination, the observed tissue damage and repressed limb regeneration. The incidence of increased injury and repressed regeneration in *C. a. bishopi* may have many contributing factors, and results from this study may serve as a platform for insight into the decline of other amphibian populations. This is the first study to profile the external microbial consortia cultured from the Cryptobranchid salamander that could play a role in the initiation or exacerbation of injuries that repress limb regeneration.

Education of Local People About Andrias japonicus *and an Introduction to the Japanese Giant Salamander Society.*

Sumio Okada

The Japanese Giant Salamander Society (JPSS) was established in 2004, and since then the Society has met annually in autumn. The JPSS meetings serve mainly to raise awareness of Japanese giant salamanders with local people and to foster the exchange of information between biologists, conservationists, civil servants and local people. The 6th annual meeting was held in 2009 at my study site in Nichinan Town, Tottori Prefecture, and over 150 people participated and several hundred local people in total were involved with supporting the meeting. I will share a brief introduction to the society, and short video of the 2009 meeting.

Tuesday, June 21- Session III: Recovery, Husbandry, and Headstarting

An update on the Ron Goellner Center for Hellbender Conservation

Chawna Schuette

This presentation will be an update on the activities and advancements of the Ron Goellner Center for Hellbender Conservation since the last hellbender symposium as well as a general overview of the centers goals and operations for those who may not be familiar. We will discuss what the focus of the center is now and what we hope to achieve in the future.

Conservation and Recovery Plan for the Eastern Hellbender in Ohio

Gregory Lipps

A total of 1487 person-hours surveying for Eastern Hellbenders in Ohio from 2006-2010 resulted in 89 observations of 79 individual hellbenders in 11 waterways. The number of hellbenders captured per person-hour searching has declined by nearly 82% in the waterways where they were previously documented in the mid-1980s, with no hellbenders detected in seven of the 15 (47%) waterways. Hellbenders were found in four waterways where they were not previously detected or where surveys were not previously performed. In seven of the 11 (64%) waterways, no individuals <45 cm were encountered, and together with the low relative abundance, the data suggests these populations are on their way to extirpation. We have recently shifted our focus in Ohio from surveying to recovery of hellbender populations. A partnership consisting of the Ohio DNR, several zoos, the Ohio EPA, local soil and water conservation districts, and a land trust are working together on multiple fronts to secure habitat where hellbenders persist, and implement strategies for ensuring multiple viable populations in the state.

Survival and Body Condition of Captive-Reared Juvenile Ozark Hellbenders (Cryptobranchus alleganiensis bishopi) Following Translocation to the Wild

Catherine M. Bodinof, Jeffrey T. Briggler, Randall E. Junge, Tony Mong, Jeff Beringer, Mark D. Wanner, Chawna D. Schuette, Jeff Ettling, Joshua J. Millspaugh

In response to Hellbender salamander (*Cryptobranchus alleganiensis*) population declines management agencies are considering translocation of captive-reared individuals to augment wild populations. We used radiotelemetry and periodic recapture to monitor survival and body condition of 36 captive-reared Ozark Hellbenders (*C. a. bishopi*) released at 2 sites on the North Fork of the White River, Missouri, from May 2008 to August 2009. At the end of our study 16 salamanders were alive, 13 had died, and the fate of 7 could not be determined. Captive-reared Hellbenders released at a site with densely arranged boulders exhibited approximately 1.5-fold higher annual survival (0.7467; daily survival = 0.9992 ± 0.0004 95% CI]) than hellbenders released at a site where boulders were patchily distributed (0.4816; daily survival = 0.9980 ± 0.0007 95% CI). At the end of the study, the mean proportional increase in body mass since release by Hellbenders at the dense boulder site (0.70 ± 0.09 SE; n =8) was 14 times greater than for Hellbenders at the patchy boulder site (0.05 ± 0.09 SE; n = 7). Hellbenders at the patchy boulder site also accrued more physical injuries and leech parasites than salamanders released at the dense boulder site. Three of the 15 Hellbenders recaptured at the end of the study tested positive for the fungus *Batrachochytrium dendrobatidis*. A 'site only' model of survival was most supported, though additional supported models suggested increased mass at release may have positively influenced survival. While more work is needed to determine the impact of translocation on long-term population dynamics of Ozark hellbenders, our study demonstrated that about half of a translocated population of captive-reared Hellbenders can survive and increased in mass in their first year post-release, given release sites are well selected.

Hellbender sperm on ice

McGinnity D, Nofs S, Kirk M, Agnews D, Carlton C, Figiel C. (US team), Browne RK, Vercammen F. (Royal Zoological Society of Antwerp, Belgium), Uteshev VK, Shishova NR, Gakhova EN (Institute of Cell Biophysics, Russia), Mansour N. (Cologne Zoo, Germany), Wu M, Li H (Peoples Republic of China), Schaftenaar W. (Rotterdam Zoo, Netherlands), Good Zoo at Oglebay, et al.

No substantial recruitment has occurred in some hellbender genetic sub-populations (evolutionary significant units or distinctive population segments) for decades. Conservation Breeding Programs (CBPs) are needed for these sub-populations and gene banking for the economical perpetuation of their genetic variation. Sperm cryopreservation provides a means to perpetuate the genetic variation of hellbenders and future restocking of these populations with genetically competent individuals. An international consortium for Hellbender cryobiology and reproduction technologies lead by Nashville Zoo, and including Michigan State University College of Veterinary Medicine, the Royal Zoological Society of Antwerp, the Institute of Cell Biophysics, Russia, Cologne Zoo, Germany, Rotterdam Zoo, Netherlands, and the US Fish and Wildlife Service, are developing techniques to sample and freeze hellbender sperm and other reproduction technologies including hormonal manipulation of reproduction. In 2009 at a time coinciding with the natural breeding season for Hellbenders, we stripped milt from hormonally induced captive hellbenders, then stored it using several several cooled (refrigerated) and cryopreservation (frozen) techniques. Sperm survived 3 days in a refrigerator and recovered motility after cryopreservation for at least six months storage. Michigan State University conducted microscopic examination, staining, and micro-videography of sperm, and the comparative structure of Hellbender sperm from 2009 utilizing electron photomicrographs. Further studies in 2010 further optimized practical techniques for the sampling of Hellbender sperm in the field and its transport, refrigerated storage, and cryopreservation. Our research also offers an opportunity to find the causes for poor reproduction of Hellbenders in nature, through the assessment of the molecular and biological characteristics of milt, sperm, gonad development, and egg quality.
The other two Cryptobranchids, the Chinese and Japanese giant salamanders are also conservation priority species. In particular in Conservation Breeding Programs the release of Chinese giant salamanders without assessment of their genetic sub-populations and the source of founders, and without knowledge of their pathogens and their screening is a major concern, and provides a precautionary example for the release of amphibians from CBPs generally. Nashville Zoo presented our pioneering conservation achievement at the International Conservation and Breeding Workshop for the Chinese Giant Salamander, Xi An, China, in June 2010. We are working further towards the establishment of global consortium for reproduction technologies for the gene banking of hellbenders and other threatened amphibians.

*Conservation of Hellbenders (*Cryptobranchus alleganiensis*) in the Ozark Highlands*

Jeffrey T. Briggler

A multi-agency, multi-discipline group consisting of researchers and biologists from several universities, public zoos, fish hatcheries, herpetologists, and state and federal agency representatives from Arkansas and Missouri has formed the Ozark Hellbender Working Group. The Ozark Hellbender Working Group pools their resources to prioritize reasons for the decline of the hellbender and coordinates recovery efforts among stakeholders. With collaboration of this working group considerable effort is underway to investigate the decline of the hellbender in the Ozark Highlands. Current recovery efforts involve monitoring populations, assessing abnormalities and diseases, exploring the effects of native and non-native fishes, assessing health conditions, reproductive hormones and heavy metal levels, determining survival and movement of released, captive-reared hellbenders, assessing sperm profiles and cryopreservation techniques, comparing hellbender genetics to determine variability and structure among populations, and continuation of captive propagation programs at the St. Louis Zoo and Shepherd of the Hills Hatchery, MDC. Overview of theses recovery efforts will be briefly discussed, as well as planning/mitigation efforts and other noteworthy highlights. The Ozark Hellbender Working Group has already played a significant role in hellbender conservation and will continue our comprehensive efforts to recovery hellbenders in the Ozark Highlands.

Wednesday, June 22- Session IV: Ecology and Behavior

Ecology of larval hellbenders in a Great Smoky Mountains stream

Kirsten Hecht-Kardasz, Michael Freake, Max A. Nickerson, and Phil Colclough

Although declines in many *Cryptobranchus alleganiensis* populations have made the species a focus of conservation and research interest, very little information is known regarding the larval stage. Knowledge of the ecology of this life stage is important for conservation and management efforts. Previous surveys uncovered a unique hellbender population in Great Smoky Mountains National Park where larval *C. alleganiensis* were regularly found. However, deep gravel beds and the associated interstitial spaces where larvae have been found in other streams are not typical at this site. Skin-diving surveys were conducted in the Little River, TN, to gather information on population structure and microhabitat use of *C. alleganiensis*. Diet samples by regurgitation were also gathered from larval hellbenders. These data were examined to look for evidence of ontogenetic shifts in diet and microhabitat use in Little River's hellbender population.

*Seasonal movements of the Japanese giant salamander (*Andrias japonicus*)— Evidence for possible breeding migration by this stream-dwelling amphibian.*

Yuki Taguchi

It is not known whether amphibian species inhabiting lotic habitats migrate to breeding sites within a stream, as migratory amphibians, such as most frogs, toads, and ambystomatid, hynobiid, and salamandrid salamanders, migrate from land to water to breed. This study examined whether breeding migration occurs in the Japanese giant salamander (*Andrias japonicus*) by analyzing features of seasonal movements and the factors that affect them. Over a 1-year period, 200 individuals from the Hatsuka River in Hyogo Prefecture were tracked in a mark-recapture study. The significant effects of the variable date on the distance of movement were confirmed in the generalized additive model. Seasonal movements were analyzed by comparing the distances moved between the pre-, post-, and nonbreeding seasons. *Andrias japonicus* moved approximately 200 m upstream, on average, during the prebreeding season and moved downstream the same distance during the postbreeding season. This was based on many short-distance moving individuals and a few relatively long-distance moving individuals; the farthest distance moved was 3969 m upstream. The upstream movement in the prebreeding season is probably related to oviposition, which occurs from late August to early September. In addition, in the nonbreeding season, from winter to spring, Andrias japonicus remained in, essentially, the same site. Therefore, the seasonal movements observed in this study can be considered "breeding migration" in the stream-dwelling amphibian, the Japanese giant salamander.

Field Observations of Parental Care of Egg Masses by Male Japanese Giant Salamanders, Andrias japonicus

Sumio Okada, Yukihiro Fukuda, Etsuko Yuki, and Tamami Okada

Although it is well-documented that male parental care occurs in *Andrias japonicus* and other Cryptobranchid salamanders, no one has described how males actually care for eggs in breeding nests. Because Cryptobranchidae is a primitive family within Caudata, documenting parental care of giant salamanders may help to elucidate evolution of parental care in salamanders. We recorded parental care behavior of male *Andrias japonicus* in one natural and one artificial nest in Tottori and Hyogo Prefectures using a video camera between August and November, 2010. We recorded the den master's behavior over 84 hrs (15 days) and 28 hrs (7 days) in the natural and artificial nest,

respectively. Here we describe and categorize care behavior types, and discuss the role of male parental care for the giant salamander.

Abundance and Spatial Ecology of Eastern Hellbenders in Indiana

Nick Burgmeier, Shem Unger, Trent Sutton, and Rod Williams

Studies that combine estimates of population density with spatial ecology analyses provide valuable information for management of wildlife species, particularly those in need of immediate conservation. The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) has experienced drastic declines throughout its range. We conducted mark-recapture surveys from June 2008 – October 2008 and July 2009 – September 2009 at 35 sites within Indiana's Blue River watershed. Density was estimated at 0.06 individuals/100 m2 and catch per unit effort was 0.05 individuals/person hour throughout the entire study area. Sex ratios were significantly skewed towards males (2.6:1). No sub-adults or larvae were found and only two nests were located. To address whether our population was suffering from an Allee effect, we used radio telemetry to examine the seasonal home range, movement patterns, and habitat use of 21 adult eastern hellbenders. Individuals were located up to three times weekly from July 2008 – October 2009. Mean 100% minimum convex polygon home-range sizes were much larger than previously reported and largest during the summer. Hellbenders moved very little throughout the year (Mean = 14.1 movements/individual) and over relatively short distances (Mean = 27.5 m) to nearby shelter rocks. The majority of hellbenders were routinely located under large, flat shelter rocks; however, five individuals periodically used bedrock, downed trees, and submerged tree root masses along the riverbank. Results from the mark-recapture and spatial ecology studies revealed that the Indiana population consists of large, older-age class individuals that occur at very low densities that are largely isolated from conspecifics.

Natural History Notes on the Eastern Hellbender in West Virginia

Joe Greathouse

During surveys of the Eastern hellbender in West Virginia in 2010, a variety of samples and observations were collected from captured individuals including blood samples, fungal swabs, body measurements, presence of injuries, and prey intake. Two of these observations, presence of injuries and prey intake, yielded statistically significant results upon analysis. Forty six of 68 (67.7%) of the individuals captured were documented to have injuries consistent with those caused by other hellbenders. MANOVA and follow-up ANOVA analysis of injuries as a factor of variables including total hellbender length, prey density, water chemistry variables, and shelter abundance indicates that these injuries are strongly correlated with the total length of the individuals (F-value: 7.8984; Pr(>F): $8.327X10^{-6}$; α: 0.00). Analysis of these variables using recursive partitioning (classification and regression trees) techniques also indicates that the greatest variable associated with injuries is total length of hellbenders ($\geq$ 42.4 cm) followed by the abundance of boulder substrate types (<46% substrate composition) at the site (Error: 0.565; model misclassification rate: 0.191). Prey intake observations were collected utilizing gastric lavage techniques on all captured individuals. 78.3% of all individuals that had prey items in their stomachs had consumed crayfish; 17.4% had consumed fish; and 8.7% had consumed hellbender eggs. Sampling also revealed that no individuals (0 of 42) had any food items in their stomachs at temperatures greater than 22.7° Celsius. No captured animals had food items in their stomachs from July 8th – September 4th at any site in the state. MANOVA and follow-up ANOVA analysis of food presence in stomachs as a factor of habitat variables indicates that lack of consumption is strongly correlated with stream temperature (F-value: 84.24; Pr(>F): $2.10X10^{-13}$; α: 0.00). Analysis of these variables using recursive partitioning techniques also indicates that the greatest variable associated with lack of consumption in this species is stream

temperatures greater than 22.65° Celsius (Error: 0.217; model misclassification rate: 0.0756). This data has been confirmed in a captive setting and could be important for future management of this species in association with climate change predictions.

Wednesday, June 22- Session V: Genetics

The Search for Sex-Linked Genes in Cryptobranchid Salamanders: Genome-Wide Gene Discovery and Marker Development

Paul Hime, David Weisrock, Justin Kratovil, and Joshua Reece

Accurate diagnosis of sex is an important aspect of conservation management efforts with endangered species, both in the wild and in captivity. However, in monomorphic species sex can be difficult to determine reliably. Such is the case with cryptobranchid salamanders, including the North American hellbender. Several non-genetic methods exist for determining hellbender sex, but each has limitations and none is universally reliable. Although sex chromosomes exist in cryptobranchids, they are weakly differentiated, suggesting that only a small portion of the genome varies between males and females. This study set out to design a genetic marker diagnostic for sex in hellbenders by identifying a region of the genome unique to one sex. Amplification of candidate sex-linked loci known from other amphibians and a preliminary AFLP analysis both failed to detect sex-linkage in a panel of known-sex hellbenders. Efforts are currently underway to identify hellbender-specific candidate sex-linked genes with high-throughput transcriptome sequencing of ovary and testis tissues, followed by in-silico subtraction of shared transcripts. In addition to gonadal transcriptome profiling, we are embarking on a gene discovery project using transcriptome sequencing of a variety of tissues from several individual hellbenders. In this marker development project, we expect to recover sequence data for large numbers of expressed genes, which will provide a genomic basis for addressing a wide range of important conservation questions in hellbenders.

*Population Genetics of the Eastern Hellbender (*Cryptobanchus alleganiensis alleganiensis*) at multiple spatial scales*

Shem D. Unger and Rod N. Williams

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a large paedamorphic salamander experiencing population declines throughout its geographic range. Causes for declines include habitat destruction, degradation, and illegal harvesting. The genetic ramifications of these population declines are currently unknown. To this end, we developed a suite of 12 hyper-variable genetic markers (microsatellites) to examine levels of gene flow, genetic variation, and genetic structure at both local and regional scales. We collected 812 individuals from 47 rivers throughout 11 states from June 2008 to September 2010. Levels of genetic diversity were relatively high among all sampling locations. The number of alleles per locus ranged from 4 to 32 (mean of 8.79), while observed heterozygosity and expected heterozygosity averaged 0.812 and 0.831, respectively among populations. We detected significant genetic structure across populations (Fst values ranged from 0.0009 between rivers within a single watershed to 0.2182 between states across their range). Understanding range-wide levels of genetic variation and differentiation will enable natural resource managers to make more informed decisions and plan conservation strategies for this cryptic, protected species.

Historical and Contemporary Patterns of Genetic Connectivity among Hellbender Populations across the Tennessee Valley

Stephen Spear, Michael Freake, Eric Routman, Rebecca Johnson, and Christopher Jenkins

Hellbenders (*Cryptobranchus alleganiensis*) are currently subject to multiple environmental pressures, such as hydrologic alterations and stream degradation through siltation, that are thought to be leading to declines across their range. In addition to habitat loss through this degradation, we would expect that loss of genetic diversity is an important conservation concern as well. Our objective was to examine whether recent habitat modification has altered historic levels of gene flow. We compared genetic patterns from two mitochondrial loci (expected to reflect historical structure) with twelve microsatellite loci (expected to reflect recent structure) among and within nine sites across a broad portion of the Tennessee Valley in Tennessee and North Carolina. We found that historically, hellbenders exhibited a strong isolation by stream distance pattern, especially when considering sites separated by less than 450 km. However, microsatellite DNA demonstrated a much weaker pattern of isolation by distance, which is indicative of disruption of genetic connectivity across the Tennessee drainage. Within sites, there was little evidence of population structure. Based on PCA visualization, we identified the Hiwassee River watershed as a historically divergent area for hellbenders. Consistent with the isolation by stream distance results, several sites showed increased divergence based on microsatellites as compared to the mitochondrial results. One of these sites was located within Great Smoky Mountains National Park, which indicates even protected areas may be subject to genetic isolation. Overall, our study demonstrates that despite low vagility, gene flow has been an important process at broad and fine scales in hellbenders. Thus, loss of genetic diversity due to isolation is likely an important conservation concern.

POSTERS

Conservation status of hellbenders in Alabama

George R. Cline and James Rayburn

Hellbenders are at the extreme southern limit of their distribution in northern Alabama. While once common in several watersheds, few live specimens have been collected in the last 10 years. This poster will present the results of recent Alabama surveys, discuss the potential causes for its current status, and discuss possible recovery actions.

*An Effective Method for Transporting Hellbenders (*Cryptobranchus alleganiensis*)*

Jennifer Pramuk, Valorie Titus, and Jason Wagner

The eastern hellbender is an impressively large, strictly aquatic salamander endemic to eastern North America. Although hellbenders have lungs, they are cutaneous respirators and require pristine, cool, swiftly moving streams and rivers with high levels of dissolved oxygen. The NYSDEC and the Buffalo Zoo have spearheaded an effort to develop captive rearing or headstarting efforts for the eastern hellbender in New York State. The Wildlife Conservation Society (WCS)/Bronx Zoo was presented with the opportunity to help with this effort through fostering a portion of the larvae, which will be reared in captivity for two years at the Bronx Zoo prior to their return to their natal river in western New York state. However, we had to find a safe and effective way to transport them from Buffalo New York, to the Bronx (an approximately 640 km journey) in July, the hottest month of the year with a monthly average high temperature of 85ºF (29ºC). The high oxygen needs of this species

required that we manufacture a reliable system for maintaining low temperatures and high dissolved oxygen during transport. This paper describes the method we developed to transport hellbender larvae.

The Clarion-Limestone Amphibian Research Center (CLARC) A Collaboration of Clarion University and Clarion-Limestone School District

Andrew C. Keth and Alysha Cypher

The Center for Conservation Studies is coordinating construction of the Clarion-Limestone Amphibian Research Center (CLARC) on the grounds of the Clarion-Limestone Area Schools. The facility is student-run and draws on the expertise of organizations like Amphibian Ark and their affiliates. This facility will serve as a model for other educational cooperatives and will contribute to amphibian conservation research.

Hellbender Recovery Efforts in Indiana

Bart T. Kraus, Zachary H. Olson, and Rod N. Williams

Eastern Hellbender (*Cryptobrachus alleganiensis alleganiensis*) populations have experienced precipitous declines throughout their range. In Indiana, hellbenders have been reduced to a single river within the Blue River watershed. Recent empirical data indicate that both population numbers and population densities are critically low. Moreover, telemetry data suggest hellbenders are scattered throughout the river with little spatial overlap among individuals. All hellbenders discovered within the last 20 years have been large, and presumably old, individuals. Despite years of larval sampling, no recruitment has been documented. Our ultimate goal is to increase local population densities and encourage natural reproduction within the Blue River. To achieve this goal, we are initiating two pilot studies: one focused on translocating adult hellbenders and one focused on head-starting and releasing sub-adults. The initial phase of our recovery efforts will consist of two types of translocations: intra-river and inter-river. Intra-river translocations will consist of relocating isolated singletons within the Blue River whereas inter-river translocations will consist of releasing adults from a genetically appropriate source population into the Blue River. We also plan to initiate a large headstarting program in Indiana. Nest searches will be conducted during the Fall 2011 to collect egg masses that will be transported to Purdue University for captive-rearing. Hellbenders will be head-started for two years and released at pre-determined locations within the Blue River to increase local densities.

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