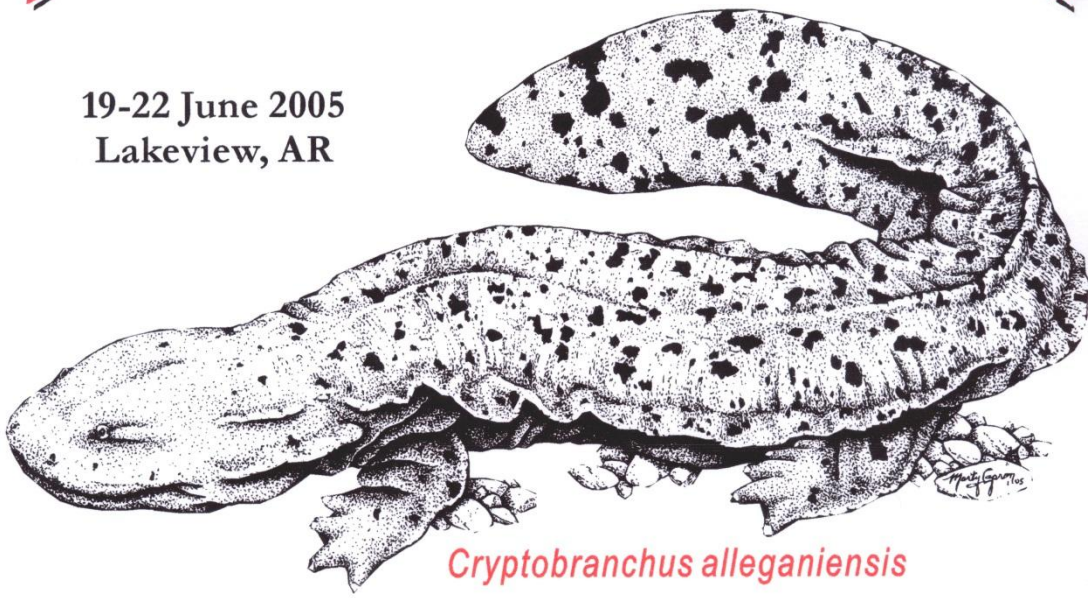
# 2nd Hellbender Symposium

19-22 June 2005
Lakeview, AR



*Cryptobranchus alleganiensis*

Co-Hosted by

Stanley E. Trauth, Benjamin A. Wheeler,
and Waylon R. Hiler

Department of Biological Sciences
Arkansas State University

# Program and Abstracts

# Welcome

We welcome all of you to the 2005 Hellbender Symposium. We are delighted to be hosting this gathering of cryptobranchid salamander enthusiasts at Gaston's White River Resort nestled within the Twin Lakes area of the Arkansas Ozarks. This second meeting brings together a wide variety of academicians, biologists, conservationists, and other interested parties who understand the need to engage in sharing ideas and concerns about these most intriguing and magnificent animals. We wish everyone a very enjoyable meeting.

Stan Trauth, Ben Wheeler, and Waylon Hiler, Department of Biological Sciences,
Arkansas State University, Jonesboro, AR

## 2005 Hellbender Symposium - Local Arrangements Group

Several graduate students and research assistants from the Department of Biological Sciences, Arkansas State University, will help us during this meeting; they are Phillip Stewart, Bobby Neal, Joe Milanovich, Charles McDowell, Nate Stephens, Jacob Sawyer, and Joy Trauth. These individuals will be wearing **red-bordered name tags**, should you require assistance.

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# Schedule of Events

(All events will meet in the Conference Lodge)

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|---|---|
| 19 June<br>4:00-6:00 pm | Registration |
| 8:00 pm | **Welcome Address** – Dr. Stan Trauth, Arkansas State University, "*The Spring River: Humans-1; Ozark Hellbender-0*" |
| 8:30 – 11:00 pm | Social/Mixer |
| 20 June<br>8:00 am | General Assembly (Registration cont.) |
| 8:45 – | Departure to Rivers |
| 6:00 pm | Arrive Back at Gaston's Resort |
| 6:30 pm | Catered BBQ Beef/Pork |
| 8:00 pm | **Keynote Speaker** – Dr. Robert F. Wilkinson, Professor Emeritus, Southwest Missouri State University – "*History of Hellbender Research West of the Mississippi River*" |
| 9:00 – 11:00 pm | Social/Mixer |
| 21 June<br>7:30 – 8:30 am | Coffee, Juice, Donuts |
| 8:30 – 8:45 | Opening Remarks |
| 8:45 – 10:15 | **Session I: State Updates** |
| 10:15 – 10:30 | Break |
| 10:30 12:00 | **Session II: Zoological Parks/Society Updates** |
| 12:00 – 1:15 pm | Lunch |
| 1:15 – 3:15 | **Session III: Habitat/Distribution** |
| 3:15 – 3:30 | Break |
| 3:30 -5:00 | **Session IV: Reasons for Declines** |
| 6:30 – 7:30 | Catered Catfish/Chicken |
| 8:00 – 11:00 | Social/Mixer |
| 22 June<br>7:30 – 8:30 am | Coffee, Juice, Donuts |
| 8:30 – 10:15 | **Session V: Behavior and Techniques** |
| 10:15 – 10:30 | Break |
| 10:30 – 12:00 | **Session VI: Open Discussion** |
| 12:00 pm | Closing Remarks and Adjournment |

# Program

## Session I: Tuesday - 21 June 8:45 – 10:15 am

### State Updates (Moderator: Chris Phillips)

8:45 am Alabama – CLINE; Arkansas – IRWIN; Georgia – HUMPHRIES; Illinois – PHILLIPS; Indiana - WALKER; Kentucky; Maryland; Missouri – BRIGGLER; Mississippi; New York; North Carolina – HUMPHRIES; Ohio; Pennsylvania – HULSE; South Carolina – HUMPHRIES; Tennessee – FREAKE; Virginia; West Virginia - HUMPHRIES

10:15 Break

## Session II: Tuesday - 21 June 10:30 – 12:00 pm

### Zoological Park/Society Updates (Moderator: John Groves)

10:30 2005 CRYPTOBRANCHID INTEREST GROUP (CIG) UPDATE. **Jessi Krebs**

10:45 AN OVERVIEW OF THE OZARK HELLBENDER WORKING GROUP. **Jeffrey T. Briggler**

11:00 THE HUSBANDRY OF POST LARVAE TO JUVENILE/SUB-ADULT HELLBENDERS (*CRYPTOBRANCHUS A. ALLEGENIENSIS*) IN CAPTIVITY. **Rich Collister**

11:15 Other Updates
Cincinnati Zoo; North Carolina Zoological Park; Omaha Zoo; Pittsburgh Zoo; St. Louis Zoo; Wonders of Wildlife

12:00 pm Lunch

## Session III: Tuesday - 21 June 1:15 – 3:15 pm

### Habitat/Distribution (Moderator: George Cline)

1:15 pm STATEWIDE SURVEY AND HABITAT EVALUATION OF HISTORIC HELLBENDER LOCALITIES IN GEORGIA: AN UPDATE. **W. Jeffrey Humphries** and **Megan A. Goddard**

1:30 HISTORIC DISTRIBUTION OF THE HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS*) IN ALABAMA. **George Cline** and **James Rayburn**

1:45 OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) HABITAT AND POPULATION PARAMETERS IN A RESEARCH SECTION OF NORTH FORK OF WHITE RIVER, OZARK COUNTY, MISSOURI 1969-1980. **Max A. Nickerson** and **Amber L. Pitt**

2:00 REASSESSMENT OF OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) HABITAT IN THE NORTH FORK OF WHITE RIVER, OZARK COUNTY, MISSOURI: 2004. **Amber L. Pitt** and **Max A. Nickerson**

2:15 COMPARISON OF THE REACH SCALE HABITAT CHARACTERISTICS OF HISTORIC AND CURRENT OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) LOCALITIES USING STANDARDIZED ASSESSMENT PROTOCOOLS. **Benjamin A. Wheeler, Waylon R. Hiler, Stanley E. Trauth,** and **Alan D. Christian**

2:30 IMPACT OF HABITAT FRAGMENTATION ON THE JAPANESE GIANT SALAMANDER (*ANDRIAS JAPONICUS*) IN THE TSUCHIYA RIVER, TOTTORI PREFECTURE, WESTERN HONSHU, JAPAN. **Sumio Okada**

2:45 THE EFFECTS OF FLOODING ON HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS*) POPULATIONS. **Max A. Nickerson**, **Amber L. Pitt,** and **Michelle D. Prysby**

3:15 OPEN FLOOR DISCUSSION ON HABITAT/Break

**Bold Underlined** -Denotes Presenter

## Session IV: Tuesday - 21 June 3:30 – 5:15 pm

### Reasons for Declines (Moderator: Peggy Horner)

3:30 pm MULTICENTRIC CUTANEOUS PAPILLOMA IN AN OZARK HELLBENDER, *CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI.* **John C. Harshbarger, Waylon Hiler, Benjamin A. Wheeler,** and **Stanley E. Trauth**

3:45 ABNORMALITIES IN THE OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) IN ARKANSAS: A COMPARISON BETWEEN TWO RIVERS WITH A HISTORICAL PERSPECTIVE. **Waylon R. Hiler, Benjamin A. Wheeler,** and **Stanley E. Trauth**

4:00 OPEN FLOOR DISCUSSION ON ABNORMALITIES

4:30 ATTEMPTS BY SPORTSMEN TO EXTIRPATE THE EASTERN HELLBENDER FROM NORTHCENTRAL PENNSYLVANIA STREAMS DURING THE 1930'S. **Peter J. Petokas** and **James Rogers**

4:45 WATER QUALITY, HORMONE PROFILING AND HEMATOLOGY OF OZARK HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*). **Mauricio E. Solis** and **Yue-wern Huang**

5:00 DOCUMENTING THE EXTIRPATION OF A KEYSTONE SPECIES (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*): THE SPRING RIVER STORY. **Waylon R. Hiler, Benjamin A. Wheeler,** and **Stanley E. Trauth**

5:15 VIDEO: EASTERN HELLBENDER BREEDING BEHAVIOR. **W. Jeffrey Humphries**

5:30 Adjourn

## Session V: Wednesday - 22 June 8:30 – 10:15 pm

### Techniques and Behavior (Moderator: Peter Petokas)

8:30 am USE OF THE BIOMARK® TAGGING SYSTEM ON THE OZARK HELLBENDER, *CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI* (AMPHIBIA: CAUDATA), IN NORTHERN ARKANSAS. **Benjamin A. Wheeler, Stanley E. Trauth, Waylon R. Hiler,** and **Chris T. McAllister**

8:45 EXAMINATION OF THE TROPHIC INTERACTIONS BETWEEN A SPECIES IN DECLINE, THE OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*), AND A PREY BASE COMPRISED OF MULTIPLE CRAYFISH SPECIES USING STABLE ISOTOPE ANALYSIS: A 30 YEAR STUDY EXPLORING CURRENT AND HISTORICAL HELLBENDER FOOD HABITS. **Waylon R. Hiler, Benjamin A. Wheeler, Stanley E. Trauth**, and **Alan D. Christian**

9:00 THE UTILITY OF DNA FROM FORMALIN-FIXED TISSUES IN PCR-BASED MOLECULAR ANALYSES **Nathan T. Stephens, Ronald L. Johnson, Benjamin A. Wheeler, Waylon Hiler,** and **Stanley E. Trauth**

9:15 THE OCCURRENCE AND BEHAVIOR OF EASTERN HELLBENDERS IN DEEPWATER POOLS IN NORTHCENTRAL PENNSYLVANIA. **Peter J. Petokas** and **James Rogers**

9:30 DIURNAL ACTIVITY AND INTRASPECIFIC INTERACTIONS AMONG EASTERN HELLBENDERS IN NORTH CAROLINA, WITH COMPARISONS TO A WEST VIRGINIA POPULATION. **W. Jeffrey Humphries**

9:45 RESPONSES AND NONRESPONSES OF LARVAL HELLBENDERS TO CHEMICAL CUES FROM INTRODUCED BROWN TROUT. **Alicia Mathis** and **Shem Unger**

10:00 OPEN DISCUSSION – INTRODUCTION OF EXOTIC SPECIES

10:15 Break

## Session VI: Wednesday - 22 June 10:30 – 12:00 pm

### Open Discussion (Moderator: Jeff Humphries)

10:30 am CITES: THE CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA. **Marie T. Maltese**

10:45 OPEN DISCUSSION – Distribution; Genetics; Pet Trade

11:30 Where Do We Go From Here?

12:00 Adjourn

# Program Abstracts

## Session I: Tuesday - 21 June 8:45 – 10:15 am

State Updates (No Abstracts)

## Session II: Tuesday - 21 June 10:30 – 12:00 pm

Zoological Park/Society Updates

### 2005 CRYPTOBRANCHID INTEREST GROUP (CIG) UPDATE

**Jessi Krebs**, Co-Chair Cryptobranchid Interest Group, Omaha Zoo, 3701 S.10th Street Omaha, NE 68107

Formed and supported by the Amphibian Taxon Advisory Group (ATAG) of the American Zoo and Aquarium Association (AZA) in 2003. The Mission statement of the Cryptobranchid Interest Group (CIG) is "To work towards the conservation of Cryptobranchids and Cryptobranchid habitat through cooperative program building, in-situ and ex-situ research, information exchange, public education, and political advocacy." Since its inception, the CIG has accomplished numerous goals to help fulfill its mission statement. In order to accomplish those goals, a seven-person steering committee was formed. The Fort Worth Zoo developed a brochure that included bullet point facts and contact information for education outreach. In the October 2003 issue of Reptiles Magazine a short blurb was featured with an introduction about CIG and its purpose along with contact information. T-shirts were designed in two styles and colors and sold with the CIG logo. To date, $2,700 has been raised in T-shirt sales. CIG received a $1,000 education grant from Omaha Zoo Education Department. The money was used to produce a life size giant salamander model and a graphics board to be used for "Live Links Educational Talks" in order to help generate funds by traveling to Reptile Expose and herpetological society meetings. So far, these educational talks have resulted in donations of $2,100. An AZA sponsored CIG list-serve was created and consist of professionals affiliated with zoos and aquariums, universities, and government agencies. A website, www.caudata.org/cig/ was also started to foster learning about Cryptobranchids and establish a resource for future donations. Funds raised through t-shirts sales and donations were distributed through a conservation/research grant. The 2004 grant recipients where Jeff Humphreys for his work with hellbenders in North Carolina and Sumio Okada for his monitoring project with Japanese giant salamanders. The 2005 grant recipients are to be announced. Applications for the grant can be found on the CIG website.

### AN OVERVIEW OF THE OZARK HELLBENDER WORKING GROUP

**Jeffrey T. Briggler**, Missouri State Herpetologist, Missouri Department of Conservation, Jefferson City, MO 65102

A multi-agency group consisting of scientists from several universities, public zoos, fish hatcheries, along with herpetologists and state and federal agency representatives from Arkansas and Missouri has formed the Ozark Hellbender Working Group. With collaboration of this multi-state working group

considerable effort is underway to investigate the decline of the Ozark Hellbender. Current recovery efforts involve conducting surveys, establishing monitoring protocols, implementing an education and outreach plan, investigating embryonic and reproductive factors, characterizing and assessing habitat, investigating water quality and agriculture runoff, examining the cause of external abnormalities, investigating the effects of non-native species (e.g., trout), and implementing a captive propagation program. The Working Group is currently writing a conservation strategy that outlines the purpose, vision, and guiding principles of the Ozark Hellbender Working Group. This document identifies actions that should be taken to address conservation and recovery of hellbenders in Missouri and Arkansas. The Ozark Hellbender Working Group has already played a significant role in hellbender conservation through education, regulation, surveys, and collaborate research effects. Future research will continue to address the appropriate projects for hellbender conservation, as well as increase awareness and appreciation with citizens and agencies. With continued efforts by the Ozark Hellbender Working Group, hellbenders will continue to inhabit Ozark rivers and streams.

### THE HUSBANDRY OF POST LARVAE TO JUVENILE/SUB-ADULT HELLBENDERS (*CRYPTOBRANCHUS A. ALLEGENIENSIS*) IN CAPTIVITY

**Rich Collister**, Wonders of Wildlife, 500 W. Sunshine St. Springfield, MO 65807

With the lack of actual experience in raising adult hellbenders from eggs and the possible future urgency in needing to restock declining populations in the wild, we set out to grow out animals to provide a baseline for husbandry protocol of hellbenders from immediate post larvae to juvenile ages. On July 30, 2003, Wonders of Wildlife received 10 post larvae eastern hellbenders to raise in captivity. These particular animals, all from the same clutch of eggs, had a hatch date of October 14, 2002, and were approximately nine months of age upon arrival. Four animals were measured on August 28th with an average total length being 116 mm. The chosen style of husbandry is direct feeding of individuals, three times per week and a passive, non-disturbed environment. Over the past two years we have seen a growth of 25% in several individuals. We will discuss Wonders of Wildlife's hellbender habitat, set up, husbandry efforts, maintenance, and growth rates. In addition, the many behaviors, interaction, and theories presented by what we have witnessed over the past two years in captivity will be discussed.

## Session III: Tuesday - 21 June 1:15 – 3:15 pm

Habitat/Distribution

### STATEWIDE SURVEY AND HABITAT EVALUATION OF HISTORIC HELLBENDER LOCALITIES IN GEORGIA: AN UPDATE

**W. Jeffrey Humphries** and **Megan A. Goddard**, Clemson University, Department of Forestry and Natural Resources, Clemson, SC 29634

The Hellbender (*Cryptobranchus alleganiensis*) has experienced drastic declines in other parts of its range, but the current status of Hellbenders in Georgia is unknown. Much of the land in the north Georgia mountains is managed by USDA Forest Service, but rapid development of private land in some watersheds is likely to threaten stream quality in the future. Hellbenders are historically known from 20

different streams (including an impounded lake) in Georgia, all within the Tennessee River drainage. Nine records are from 1970 or earlier, and only five of the records are from the last 10 years. In addition, sufficient anecdotal evidence suggests that Hellbenders may also occur in the Chattooga River drainage, which is presumed to be outside of their range. During 2005, I am re-surveying all known Hellbender localities by diurnal snorkeling and rock turning to determine relative abundance. In addition, habitat quality is being evaluated using EPA protocols and watershed land use data will be analyzed using GIS. By combining relative abundance of Hellbenders and habitat quality and land use data, predictive models will be developed to explain the current distribution and relative abundance of Hellbenders in Georgia. In addition to predictive modeling, substantial effort will be put into determining the validity of reports of Hellbenders in the Chattooga River drainage.

## HISTORIC DISTRIBUTION OF THE HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS*) IN ALABAMA

**George Cline** and **James Rayburn**, Biology Dept., Jacksonville State University, 700 Pelham Road North, Jacksonville, AL 36265-1602

Hellbenders (*Cryptobranchus alleganiensis*) are large aquatic salamanders found in clear, fast flowing streams in the eastern United States. The bulk of the species distribution centers around the Ohio and Susquehanna River systems, but isolated populations occur in southern Missouri and northern Arkansas. Recent presentations have indicated that hellbender populations are declining throughout their range, but current data are lacking in most states. In Alabama, hellbenders are restricted to the Tennessee River drainage in the northern 20% of the state. Museum records for hellbenders are limited. Preliminary review of museum records has revealed 18 specimens from 5 counties. The bulk of the distribution is limited to 3 watersheds in these counties. Interestingly, these collections are largely limited to a 10-year period ranging from 1963-1973. The oldest specimen discovered at this time is from 1928. Reports of this species from colleagues at other Universities are as recent as 2004, but no large populations have been documented. Historic distributions are linked to substrate types. In Alabama, hellbenders appear to associate with limestone rock.

## OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) HABITAT AND POPULATION PARAMETERS IN A RESEARCH SECTION OF NORTH FORK OF WHITE RIVER, OZARK COUNTY, MISSOURI 1969-1980

**Max A. Nickerson** and **Amber L. Pitt**, University of Florida, Florida Museum of Natural History, P.O. Box 117800, Gainesville, FL 32611-7800

Habitat data from the North Fork of White River, Ozark County, Missouri from 1969-1980 include year-round water quality, temperature, stream flow, benthic samples, *Cottus hypselurus* and *C. carolinae*, food studies. Additional data include observations from annual stream surveys including diurnal and nocturnal ichthyofauna samples and observations, population estimates and population structure for *C. a. bishopi* and turtles, relative frequency of crayfish observations, scientific and illegal harvesting, nesting sites, human activity, stream bottom substrate, and landscapes. These data form a baseline which we compare to those obtained in 2004.

## REASSESSMENT OF OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) HABITAT IN THE NORTH FORK OF WHITE RIVER, OZARK COUNTY, MISSOURI: 2004

**Amber L. Pitt** and **Max A. Nickerson**, University of Florida, Florida Museum of Natural History, P.O. Box 117800, Gainesville, FL 32611-7800

Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) habitat data from the North Fork of White River, Ozark County, Missouri was reassessed in 2004. Parameters examined include water quality, temperature, substrate distribution, benthic composition, and recreational use of the river. General health observations of hellbenders, changes in community composition, and alterations of hellbender nesting sites since 1980 are discussed.

## COMPARISON OF THE REACH SCALE HABITAT CHARACTERISTICS OF HISTORIC AND CURRENT OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) LOCALITIES USING STANDARDIZED ASSESSMENT PROTOCOOLS

**Benjamin A. Wheeler**[1], **Waylon R. Hiler**[2], **Stanley E. Trauth**[2], and **Alan D. Christian**[2],
[1]Environmental Sciences Program, Arkansas State University, P.O. Box 847, State University, AR 7467;
[2]Department of Biological Sciences, Arkansas State University, P.O. Box 599, State University, AR 72467-0599

Habitat degradation is typically cited as a reason for declines in Ozark hellbender populations. While habitat degradation is evident, many sites appear to contain suitable microhabitat, but do not support hellbender populations. We used three standardized protocols, US EPA Rapid Bioassessment Protocol (RBP), Ohio Qualitative Habitat Evaluation Index (QHEI), and Basin Area Stream Survey (BASS), to compare reach-scale habitat at nine locations within each of the Eleven Point (EP) and Spring (SR) rivers in the eastern Ozark Mountains. Sites were divided into Historically Present (HP), Currently Present (CP), and Reference Reaches (RR). Although EP sites scored consistently higher than SR sites for the RBP and QHEI, all sites scored close to the optimal levels. The BASS data were analyzed using PCA, and three resulting axes explained 52.8% of the variation. ANOVA of the PCA loading scores indicated significant differences between the rivers and between SRCP sites and both EPCP and EPHP sites. Parameters most associated with SR sites were rooted vegetation and embeddedness, whereas woody debris and bank cover were associated with EP stations. Our results suggest the Spring River is suffering from loss of riparian zones, thus, resulting in the degradation of in-stream habitat.

## IMPACT OF HABITAT FRAGMENTATION ON THE JAPANESE GIANT SALAMANDER (*ANDRIAS JAPONICUS*) IN THE TSUCHIYA RIVER, TOTTORI PREFECTURE, WESTERN HONSHU, JAPAN

**Sumio Okada**, Division of Environmental Biology, Shimane University, Matsue 690-8504 Japan

I have been investigated ecology and population status of *A. japonicus* in the Tsuchiya River since 2001. Study site was headwaters and consisted of nearly undisturbed habitat. The lower reaches of study site

runs through village, and it was separated sections by dams and cement walls. It is believed that alteration of the species' habitat by dams and bank protection walls has contributed to population declines. However, there has been no study that tried to assess the impact of habitat fragmentation on giant salamander populations. I conducted preliminary survey of relationships between habitat fragmentation and giant salamander population in the lower reaches of study site, 2004. A total 51 individuals were captured from the lower section and they were mostly only large adult. Forty individuals (78%) were over 60 cm (TL) and only two individuals (4%) were less than 40cm (TL), whereas multiple sized salamanders were captured from the upper section. More than twenty salamanders tried to climb the dam and failed during breeding season. Two females deposit their eggs under concrete floor beside the dam, but all eggs flowed out of the concrete nest. It might be inferred from these observations that the dam prevented the breeding migration of *A. japonicus* and small individuals were relatively low recruitment in the lower section.

### THE EFFECTS OF FLOODING ON HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS*) POPULATIONS

**Max A. Nickerson, Amber L. Pitt,** and **Michelle D. Prysby**[1], University of Florida , Florida Museum of Natural History, Gainesville, FL 32611-7800; [1]Great Smoky Mountain Institute at Tremont, Townsend, TN 37882

The effects of flooding on *Cryptobranchus alleganiensis* in two river systems (North Fork of White River, MO and Little River, TN), were examined using relocation of tagged individuals and time required to capture individuals following flooding. Differences in the streams benthic structure, relief, volume of water, and visible changes in riverine habitat are discussed in relation to *C. alleganensis* data following two major floods.

## Session IV: Tuesday - 21 June 3:30 – 5:15 pm

Reasons for Declines

### MULTICENTRIC CUTANEOUS PAPILLOMA IN AN OZARK HELLBENDER, *CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*

**John C. Harshbarger[1], Waylon Hiler, Benjamin A. Wheeler**, and **Stanley E. Trauth**, [1]Department of Pathology, George Washington University Medical Center, Washington, DC, 20037; Department of Biological Sciences, Arkansas State University, P.O. Box 599, State University, AR 72467-0599

An Ozark Hellbender that died in captivity had raised, bosselated, lesions scattered singly and in clusters on the skin of the tail and trunk. These protrusions ranged up to 2 cm in greatest dimension. Pigmentation decreased as lesion size increased. Small lesions were skin colored. The largest lesions were pink and underlying blood vessels were apparent. None was ulcerated. Microscopically the epidermis was thickened by cellular proliferation that abnormally increased cell layers. Due to this thickening process pegs of epidermis extended into the stratum spongiosum of the dermis where fibro-vascular dermal papillae interdigitated with the epidermal pegs. Pegs consisted centrally of squamous cells and were bordered distally by a layer of basal cells. A basement membrane derived from the

dermal fibrous tissue separated the basal cell layer from the normal dermis. The basement membrane appeared intact in all locations, ruling out invasion by this multicentric papillary epidermal neoplasm. Mitotic figures were rarely seen indicating a slow rate of growth. This epidermal papilloma complements a papillary squamous-cell carcinoma previously described in another Ozark hellbender.

## ABNORMALITIES IN THE OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*) IN ARKANSAS: A COMPARISON BETWEEN TWO RIVERS WITH A HISTORICAL PERSPECTIVE

**Waylon R. Hiler[1], Benjamin A. Wheeler[2], and Stanley E. Trauth[1],** [1]Department of Biological Sciences, Arkansas State University P.O. Box 599, [2]Environmental Sciences Ph.D. Program, Arkansas State University, P.O. Box 847, State University, AR 72467

We documented abnormalities within Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) populations in the Eleven Point River (Randolph County, Arkansas) and the Spring River (Fulton County, Arkansas) as part of ongoing monitoring efforts in this species. We found abnormalities in 90% (9 of 10) and 40% (36 of 97) of hellbenders in the Spring River and Eleven Point River, respectively, during the 2003-2004 field seasons. Most abnormalities found in Eleven Point hellbenders were generally less pronounced and seemed to be more intrinsic to the species' natural history (i.e., vicissitudes of living), whereas those found in Spring River hellbenders were gross morphological aberrations. In addition, we compared the type and rate of observed abnormalities with those found in museum vouchers collected from the Spring River between 1970 and 1975. We observed abnormalities in 12.5% of the museum specimens collected from our Spring River localities. These rates are much higher than previously reported for hellbenders. The increase in the abnormality rate appears to be concurrent with the documented population decline observed in the Spring River. Our study not only illustrates an increasing trend of hellbenders exhibiting unusual morphological conditions (e.g., epidermal papillomas, extreme abrasions/lacerations, fungal infections, etc.), but also stresses the need for meticulous gathering of field data. The causes of hellbender abnormalities remain speculative; however; plausible explanations may be related to intraspecific interactions, heavy anthropogenic interactions with the microhabitat, viral infections, non-point/point source pollution, and the preponderance of old-aged individuals. Finally, our findings emphasize the need for a proactive conservation effort within this species.

## ATTEMPTS BY SPORTSMEN TO EXTIRPATE THE EASTERN HELLBENDER FROM NORTHCENTRAL PENNSYLVANIA STREAMS DURING THE 1930'S

**Peter J. Petokas** and **James Rogers**, Department of Biology, Lycoming College, Williamsport, PA 17701

Sportsmen's associations, with the support of the Pennsylvania Fish Commission, conducted a "War on Waterdogs" during the 1930's in tributaries of the Susquehanna River West Branch in Northcentral Pennsylvania. Details of planned and completed hunts were published in local newspapers along with photographs of sportsmen and slaughtered hellbenders. Sportsmen seemed to believe that hellbenders were depredating trout populations and based this belief on purported observations of small trout and

trout eggs found inside hellbender stomachs. Hellbender hunts were conducted late at night and involved the use of wire traps, outlines, and gigs. Some published reports contain specific locality information, but most contain only the name of the stream or streams were hunts took place. Most hellbender hunting was done by wading, but some sportsmen used boats equipped with automobile headlamps powered by batteries. One hunt yielded 160 hellbenders, of which 70 were from "one hole at one time." Other hunts yielded few or no hellbenders. As many as 50 sportsmen participated on some hellbender hunts. One sportsmen's club paid bounties on "nearly 800" hellbenders captured during a single month. Hellbenders still occur in most streams where hellbenders were slaughtered during the 1930's and we are unaware of any additional organized hellbender hunts having been undertaken in the 6-1/2 decades since then.

## WATER QUALITY, HORMONE PROFILING AND HEMATOLOGY OF OZARK HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*)

**Mauricio E. Solis** and **Yue-wern Huang,** Department of Biological Sciences, 105 Schrenk Hall, 1870 Miner Circle, University of Missouri-Rolla, Rolla, MO 65409

Ozark hellbenders were collected from the North Fork of the White River and the Eleven Point River in Missouri to evaluate their health and hormone status. Blood samples were analyzed for twenty five hematologic and serum chemistry parameters in 33 Ozark hellbenders in Missouri and compared against the data from the Eastern hellbenders in the southeast United States. Hormone profiles were analyzed in 42 Ozark Hellbenders from our Missouri collecting sites. Water quality and nutrient levels were analyzed on the sites where the Ozark Hellbenders were collected. The results showed that the majority of the blood parameters analyzed were similar between the same sexes of these two subspecies, though a few significant differences were identified. Assuming that Eastern hellbenders were healthy according to their size classes, the Ozark hellbenders in the North Fork of the White River and Eleven Point River might be considered as healthy within the context of the parameters measured. Additionally, approximately 81% of the Ozark hellbenders captured were likely to be at least or more than 25 years old. This age conditions raise our concern over their health status in the near future, particularly on Ozark hellbenders. Hormone data are still being analyzed. Water quality data indicated the presence of 12 chemicals (pesticides, plasticizers, herbicides, estrogens). The levels of these chemicals were below the EPA standards for water quality criteria in aquatic environments, though species-specific sensitivity should be considered.

## DOCUMENTING THE EXTIRPATION OF A KEYSTONE SPECIES (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*): THE SPRING RIVER STORY

**Waylon R. Hiler**[1], **Benjamin A. Wheeler[2],** and **Stanley E. Trauth[1],** [1]Department of Biological Sciences, Arkansas State University, P.O. Box 599; [2]Environmental Sciences Program, Arkansas State University, P.O. Box 847, State University, AR 72467

Since the early 1980's the Ozark hellbender, North America's largest salamander, has undergone a series of population declines throughout its entire range. Ozark hellbenders from the Spring River (Fulton County, Arkansas) have undergone the most drastic population decline witnessed within their

range over the past 20 years. We surveyed the entire Spring River from the spring head to the Arkansas Game and Fish Commission's Hardy Beach Access. Any hellbenders caught were removed from the river and placed in a raceway at the Mammoth Spring National Fish Hatchery in a cooperative captive propagation effort with the U.S. Fish and Wildlife Service, the Arkansas Game and Fish Commission, and Arkansas State University. We also gathered historic data sets from the past 35 years, which illustrate a drastic shift in size classes toward larger animals. This is a characteristic thought to be indicative of a population exhibiting a lack of recruitment.

## Session V: Wednesday - 22 June 8:30 – 10:15 pm

Techniques and Behavior

### USE OF THE BIOMARK® TAGGING SYSTEM ON THE OZARK HELLBENDER, *CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI* (AMPHIBIA: CAUDATA), IN NORTHERN ARKANSAS

**Benjamin A. Wheeler**[1], **Stanley E. Trauth**[2], **Waylon R. Hiler**[2], and **Chris T. McAllister**[3].
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The Ozark hellbender, *Cryptobranchus alleganiensis bishopi*, is a robust salamander restricted to streams of the Ozark Plateau of southern Missouri and northern Arkansas. In 2001, *C. a. bishopi* was listed as an endangered species candidate, as populations appear to be declining in streams where this salamander was once common. The possible reasons for the decline range from overcollection to various types of habitat alteration. Currently, the only method available for monitoring these limited populations involves searching for hidden animals by flipping boulders while SCUBA diving; this poses a risk to the hellbender and potentially alters/destroys habitat or nesting sites. We conducted a preliminary study of the Ozark hellbender on the Eleven Point River (Randolph County, Arkansas) using the Biomark® tagging system and underwater antenna (Biomark Inc., Boise, Idaho). On 22 July 2004, six hellbenders were collected and tagged with a 134.2 kHz ISO PIT (Passive Integrated Transponder) tag and released at the collection site. The following day, we returned to the location and attempted to detect the tagged hellbenders from a slowly-moving jon boat. Within a search period of two hours, we were successful in locating two of the six (33%) tagged hellbenders. For the first time, this study suggests that hellbenders can be PIT tagged and located with a transceiver while in their natural habitat, making handling and habitat disruption minimal for future population studies.

### EXAMINATION OF THE TROPHIC INTERACTIONS BETWEEN A SPECIES IN DECLINE, THE OZARK HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS BISHOPI*), AND A PREY BASE COMPRISED OF MULTIPLE CRAYFISH SPECIES USING STABLE ISOTOPE ANALYSIS: A 30 YEAR STUDY EXPLORING CURRENT AND HISTORICAL HELLBENDER FOOD HABITS

**Waylon R. Hiler**[1], **Benjamin A. Wheeler**[2], **Stanley E. Trauth**[1], and **Alan D. Christian**[1], [1]Department of Biological Sciences, Arkansas State University, P.O. Box 599, State University, AR 72467;

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Since the early 1980's the Ozark hellbender (*Cryptobranchus alleganiensis bishopi*), North America's largest salamander, has undergone a series of declines throughout its entire range. Two Arkansas rivers which hellbenders are known to inhabit are the Eleven Point River (Randolph County, Arkansas) and the Spring River (Fulton County, Arkansas). Ozark hellbenders from the Spring River have undergone the most drastic population decline witnessed within their range over the past 20 years. Shifts in benthic habitat and community compositions could influence these declines, which in turn, could affect the species composition of their primary prey item, the crayfish. The objectives of our study were to use stable C and N isotope analysis to 1) determine what individual or multiple species of crayfish contribute to hellbender diets, 2) determine if certain size classes of crayfish dominate hellbender diets, 3) establish whether predatory opportunism persists, decreases, or increases throughout a hellbenders lifetime, and 4) explore the potential of using amphibian museum specimens to compare historic versus current food habits. By linking stable C and N ratios results and relative abundances of crayfish in each of the river sites, we quantified species-specific trophic relationships between hellbenders and crayfish. Our results suggest hellbenders are not selecting for one species or size class of crayfish, and that multiple species of crayfish contribute to long-term diet patterns of the hellbender. Also, we found increasing $\delta^{15}$ N and $\delta^{13}$C values to be correlated with increasing total lengths, indicating a clear shift in diet patterns with age. Furthermore, tissue samples analyzed from hellbenders collected in the early 1970's appear to coincide with current hellbender $\delta^{15}$ N and $\delta^{13}$C values.

## THE UTILITY OF DNA FROM FORMALIN-FIXED TISSUES IN PCR-BASED MOLECULAR ANALYSES

**Nathan T. Stephens[1], Ronald L. Johnson[1], Benjamin A. Wheeler[2], Waylon Hiler[1],** and **Stanley E. Trauth[1]**, [1]Department of Biological Sciences, Arkansas State University, P.O. Box 599, State University, AR 72467; [2]Environmental Sciences Ph.D. Program, P.O. Box 847, State University, AR 72467-0599

Total DNA samples were isolated from formalin-fixed museum specimens to evaluate the quality of DNA from fixed tissues. We compared two extraction methods for overall DNA yield and used two methods of PCR to amplify small DNA fragments. Muscle tissue was taken from a wood frog, *Rana sylvatica*, and a hellbender salamander, *Cryptobranchus alleganiensis*. These animals had been fixed in formalin for an indeterminate amount of time, stored in 70% ethanol, and catalogued into museum collections. One extraction method lysed tissues for an extended period of time in a buffered solution of proteinase K and dithioerythritol (DTE) prior to multiple organic extractions. The second method included a wash of tissues with a potassium phosphate solution prior to silica membrane extraction (DNeasy®). Total DNA samples were quantified using UV spectroscopy and agarose gel electrophoresis. Wood frog DNA was amplified using RAPD primers and microsatellite primers specifically designed for the species. Hellbender DNA was amplified using RAPD primers. The amplified products were observed by agarose gel electrophoresis. Fragment size was determined by comparison to a molecular size standard and concentrations were estimated by relative band intensity. These data were used to determine the quality and applicability of DNA from formalin fixed tissues to molecular studies using PCR-based methods.

## THE OCCURRENCE AND BEHAVIOR OF EASTERN HELLBENDERS IN DEEPWATER POOLS IN NORTHCENTRAL PENNSYLVANIA

**Peter J. Petokas** and **James Rogers**, Department of Biology, Lycoming College, Williamsport, PA 17701

Some tributaries of the Susquehanna River West Branch in Northcentral Pennsylvania contain deepwater pools ranging from 5-10 meters in depth. Loyalsock Creek in Lycoming County contains several deepwater pools frequented by SCUBA divers. Divers commonly report sightings of one or two active adult hellbenders during night dives. However, underwater surveys are often futile with few or no hellbenders found beneath large stone slabs, submerged logs, or in crevices on underwater ledges. Much of the bottom of the deepwater pool (maximum depth = 10 meters) at Slabtown was covered with a thick layer of sand and silt prior to September 2004 when three tropical storms dumped heavy rainfall on Pennsylvania. The high discharge from the storms moved large amounts of cobble and rubble into the deepwater pool, dramatically changing the benthic habitat. Beginning in early October, 2004, dives at Slabtown revealed many juveniles and small adults inside piles of loose stone and we presume that the hellbenders were carried into the deepwater pool by the same flows that moved the cobble. During night dives, we observed hellbenders with their heads protruding through the cobble, but when capture was attempted they retreated deep into the piles of loose stone. By late October, hellbender sightings were few and none were seen during our last dive on the night of October 29, 2004.

## DIURNAL ACTIVITY AND INTRASPECIFIC INTERACTIONS AMONG EASTERN HELLBENDERS IN NORTH CAROLINA, WITH COMPARISONS TO A WEST VIRGINIA POPULATION

**W. Jeffrey Humphries**, Clemson University, Department of Forestry and Natural Resources, Clemson, SC 29634

I studied a population of hellbenders in West Virginia (WV) during 1998-2000 and have been studying a population in western North Carolina (NC) since 2003. Forty-four individuals were marked in WV and 83 have been marked in NC. Both populations were similar in density and population structure, but several life history attributes varied markedly. Whereas WV hellbenders were highly active at night, especially in early summer, NC hellbenders were highly diurnal and were difficult to find at night. Outside of the nesting season, hellbenders in NC were most diurnally active during May, with over five individuals observed per hour. During early September, an average of 12 hellbenders was observed per hour during diurnal surveys, mainly consisting of large aggregations near a single nesting rock. Injury rates (missing toes, limbs, eyes, or having bite scars) also differed between the two populations. In NC, 77% of individuals were injured compared to 49% in WV. Males had a higher rate of missing legs and head bites from conspecifics compared to females in NC, but conspecific bite marks on the body and tail were similar between males and females. Geographic differences in activity and injury rates are difficult to explain, but may reflect variability in resources (e.g., prey abundance, shelter, nesting habitat) between the study sites.

**RESPONSES AND NONRESPONSES OF LARVAL HELLBENDERS TO CHEMICAL CUES FROM INTRODUCED BROWN TROUT**

**Alicia Mathis** and **Shem Unger**, Southwest Missouri State University, Springfield, Missouri 65804

Introduced species often have negative effects on native fauna. In Missouri, where hellbenders (*C. a. alleganiensis* and *C. a. bishopi)* have experienced substantial population declines, both brown and rainbow (trout have been widely introduced into rivers across the state. Because there are no native trout in Missouri, local hellbenders have not had the opportunity to evolve anti-trout defenses. Brown and rainbow trout also have been introduced into the hellbender habitat in the eastern U.S.A., but native trout (brook trout, *Salvelinus fontinalis*) also occur in these streams. We collected three clutches of hellbenders, two from Missouri (one of each subspecies) and one from North Carolina (*C. a. allenganiensis*) and reared them in the laboratory. We tested whether larvae from each clutch responded to chemical cues from brown trout (*Salmo trutta*) with antipredator behavior. Larvae from the North Carolina clutch exhibited a significant decrease in activity (a common antipredator response) following exposure to trout cues, but did not change activity in response to chemical cues from a nonpredatory species (stonerollers). In contrast, larvae from both clutches collected in Missouri did not distinguish between stimuli from trout and stonerollers. We suggest that the potential impact of trout predation on larval hellbenders should receive further study.

## Session VI: Wednesday - 22 June 10:30 – 12:00 pm

Open Discussion

**CITES: THE CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA**

**Marie T. Maltese**, Biologist, USFWS, Division of Scientific Authority, 4401 N. Fairfax Dr. Rm. 750, Arlington, VA 22203

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is a treaty of 167 member nations which manages the international trade of species of conservation concern. Species are listed in one of three Appendices within CITES, depending on the level of concern necessary for the survival of the species. Appendix I is the most restrictive of the appendices; these species may not be traded for commercial purposes, and require both an export permit from the country of origin and an import permit from the receiving country. Examples of Appendix I species include: elephants, tigers, and many tropical bird species.

Most CITES-listed species are found within Appendix II. Species that include sturgeon, bobcats, and many reptiles in the pet trade, these specimens can be traded commercially, and require the issuance of an export, or re-export, permit to be shipped outside of a country's borders. Appendix III is the least restrictive of the three appendices, yet it still requires that trade data be maintained, similar to all of the CITES Appendices. A country may unilaterally list a species within Appendix III in order to institute the need for the issuance of a certificate of origin for export purposes, and hence, the collection of trade data. Appendix III listings are often undertaken by a country to gain valuable data regarding the international trade of its native flora and fauna.

The United States is considering listing the hellbender in Appendix III because of continuing reports of the species' decline and increased collection of live specimens for the international pet trade. I will be attending the symposium to listen to the speakers and meet the researchers and managers who are directly involved in the conservation of the species. I look forward to meeting everyone, and am very much interested in learning as much as I can about the status of our native cryptobranchids.

# Author Index

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