

**Endangered Species
Section 6 Grant Proposal**

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**Evaluation of Health Conditions, Reproductive Hormones, and Mercury in Adult
Hellbenders (*Cryptobranchus alleganiensis*)
E-1-45**

01 July 2006 - 30 June 2007

Missouri Department of Conservation

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Date Prepared:

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(Univ. Missouri-Rolla)

TITLE: Evaluation of Health Conditions, Reproductive Hormones, and Mercury in Adult Hellbenders (*Cryptobranchus alleganiensis*)

EXPECTED DURATION: One Year: 01 July 2006-30 June 2007

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I. NEED

Both the eastern (*Cryptobranchus alleganiensis alleganiensis*) and Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) have experienced marked population declines. Data reveal a shift in age structure of hellbender populations, with larger, mature individuals being most prevalent and young age classes being virtually absent (Wheeler et al. 2003). With the decline of hellbenders and a shift in age structure over the years, much information is needed on the general health conditions of this aging population of hellbenders. Preliminary information has been provided by Dr. Yue-wern Huang (University of Missouri-Rolla) regarding hematology and serum chemistry (Solis and Huang, 2005a), reproductive hormones (Solis and Huang, 2005b), and chemical and nutrient assessments (Huang, 2005). This research provided much insight as to the next steps and directions needed to help recover hellbenders in Missouri. Investigating and understanding the health conditions and reproductive hormones in hellbenders is important in assessing if this aging population can successfully reproduce in the wild across Missouri's rivers, as well as determine the feasibility of removing wild captured hellbenders for long-term propagation efforts.

II. RECOVERY STATUS/PRIORITY

Currently, two subspecies (Ozark and eastern hellbenders) are found in 17 states. The Ozark hellbender is known to only occur in Missouri and Arkansas. Missouri is the only state that has both Ozark and eastern hellbenders. The Ozark hellbender is a candidate for federal listing under the Endangered Species Act with federal recovery priority number of three. The eastern hellbender status review document has been submitted to U.S. Fish and Wildlife Service and is currently being evaluated for federal listing (Mayasich and Phillips, 2003). Both subspecies are listed as critically imperiled and state endangered in Missouri.

IV. PROJECT OBJECTIVES

- 1) Determine general health conditions (hematology and serum chemistry) of wild adult hellbender throughout Missouri.
- 2) Determine if male and female adult hellbenders on lower North Fork River are reproductively fit (reproductive hormones).

- 3) Determine if male and female adult hellbenders used for captive propagation programs are reproductively fit (reproductive hormones).
- 4) Determine if mercury accumulations are occurring in adult hellbenders.
- 5) Determine if there is a link between health condition and abnormalities.

IV. EXPECTED RESULTS OR BENEFITS

The information gained from this project will provide a general overview of the health and reproductive conditions and potential mercury accumulations of an aging population of hellbenders. In addition to obtaining general health data, comprehensive database for normal range of each blood parameter will be established. This project will increase our knowledge of the breeding conditions of hellbenders in the wild, provide information on the potential of mercury accumulations in hellbenders, provide insight into hellbender abnormalities, and provide useful information needed for captive propagation protocols. This project will address the question of whether hellbenders removed from the wild and placed in captivity for propagation are reproductively active based upon hormone levels.

This project will address the following recovery plan priority activities as identified in the draft *Ozark Hellbender Conservation Strategy: An action plan for the recovery of the Ozark hellbender*, prepared by the Ozark Hellbender Working Group and *Missouri Department of Conservation 5 year T&E action plan* prepared by Missouri Department of Conservation species experts.

- Health and reproductive conditions
- Captive propagation protocols and feasibility
- Abnormalities
- Water Quality (Mercury)

V. PROJECT APPROACH

While conducting annual MDC hellbender surveys, 4 ml blood samples will be removed from each adult hellbender > 400 g. Dr. Randy Junge of the St. Louis Zoo recommended that one ml of blood could be removed for every 100 g of body weight without harming the specimen (Wright and Whitaker, 2001). Blood will be drawn from the caudal vein slightly posterior of the cloaca. The blood will be placed in heparinized vacutainers, and centrifuged at 300 g for 10 minutes. The plasma will be stored in liquid nitrogen at the University of Missouri, Rolla until bioassays are performed. Plasma samples will be sent and analyzed at Antech Diagnostics Laboratory in Memphis, Tennessee and University of Missouri, Rolla lab facilities of Dr. Yue-wern Huang.

Blood analysis involving health condition (Appendix 1) of hellbenders will be taken and analyzed for all hellbenders captured throughout targeted rivers for Missouri Department of Conservation surveys efforts in 2006. The Niangua, Gasconade, and Meramec rivers will be the primary focus with scattered surveys on other rivers with both eastern and Ozark hellbenders. In addition, blood samples from one location on the lower North Fork will be taken on a monthly basis for a 3 month period (July, August, and September) for both health condition analysis and reproductive hormone analysis (Appendix 1). Mercury has been identified to be a contaminant of concern in some of these water bodies based on fish accumulation data. Therefore, the blood will be screened for mercury

levels to see if high levels of accumulation are occurring in hellbenders. About 100 mg of tissue or whole blood is needed to analyze for mercury levels. These same analyses (health condition and reproductive hormones) will also be conducted on the 8 hellbenders currently being held for captive propagation at the St. Louis Zoo. Blood samples will be removed in May, July, September, and November from these captive animals.

Schedule: July 2006 through June 2007

Tasks	J	A	S	O	N	D	J	F	M	A	M	J
Collect blood samples for general health parameters, and mercury from scattered river locations	X	X	X	X								
Collect blood samples from North Fork River for health and reproductive condition	X	X	X									
Collect blood samples for captive held hellbenders for health and reproductive condition	X		X		X							
Analyze blood samples				X	X	X	X	X	X			
Final report preparation										X	X	X

VI. PROJECT LOCATION and PERSONNEL:

Surveys to collect blood samples for hellbenders will mainly focus on the Niangua River (Dallas and Laclede counties), Gasconade River (Pulaski and Phelps counties), Meramec River (Crawford County), and North Fork of White River (Ozark County) with scattered surveys on Current River (Shannon and Carter counties) and Eleven Point River (Oregon County).

The project will be completed via a cooperative agreew.eut between the Missouri Department of Conservation (MDC) and University of Missouri-Rolla (UMR). The ongoing survey for hellbenders in Missouri's rivers conducted by MDC (Dr. J ffBriggler and department staff) will facilitate collection of blood. Dr. Yue-wem Huang and students (UMR) will conduct the necessary bioassays of the blood samples.

Project Budget July 2006 - June 2007	
Costs	Amounts
Contract to University (Graduate assistantship, laboratory technician, laboratory analysis, and other supplies)	\$ 48,136 /.
Project Total	\$ 48,136 v/
MDC share (44 %)	\$ 21,136 /
FWS share (56 %)*	\$ 27,000

* Federal amount requested is \$ 27,000.

VII. LITERATURE CITED:

- Huang, Y. 2005. Chemical and nutrient assessment in Missouri River. MDC Final Report. Jefferson City, MO.
- Mayasich, J., and C. Phillips. 2003. Eastern Hellbender Status Assessment Report. USFW Report. 41 pp.
- Solis, M.E., and Y. Huang. 2005a. Hematology and serum chemistry of Ozark and Eastern Hellbenders (*Cryptobranchus alleganiensis*). MDC Final Report. Jefferson City, MO.
- Solis, M.E., and Y. Huang. 2005b. Reproductive hormones in the breeding season of Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*). MDC Final Report. Jefferson City, MO.
- Wheeler, B.A., E. Prosen, A. Mathis, and R.F. Wilkinson. 2003. Population declines of a long-lived salamander: a 20 + -year study of hellbenders, *Cryptobranchus alleganiensis*. *Biological Conservation* 109(2003): 151-156.

Appendix 1: List of blood parameters that will be analyzed for Ozark and eastern hellbenders.

Health Condition (Hematology and Serum Chemistry):

- 1) Blood parasites (present / absent)
- 2) Hematocrit (%)
- 3) WBC estimate (10^3 uL): (counts of leukocytes, erythrocytes and thrombocytes)
- 4) Heterophils / Polymorphonuclearcytes
- 5) Absolute neutrophils
- 6) Lymphocytes(%)
- 7) Absolute lymphocytes
- 8) Monocytes (%)
- 9) Absolute monocytes
- 10) Eosinophils (%)
- 11) Absolute eosinophils
- 12) Basophils (%)
- 13) Absolute basophis
- 14) Glucose (mg/dL)
- 15) Urea nitrogen (mg/dL)
- 16) Total protein (g/dL)
- 17) Albumin (g/dL)
- 18) Globulin (g/dL)
- 19) AST (U/L) (aspartate aminotransferase)
- 20) Calcium (mg/dL)
- 21) Phosphorus (mg/dL)
- 22) Sodium (mEq/L)
- 23) Potassium (mEq/L)
- 24) Chloride (mEq/L)
- 25) CPK (U/L): Creatine phosphokinase
- 26) Uric acid (mg/dL)
- 27) Mercury

Reproductive Hormones (Serum analysis):

- 1) Estradiol (ng/mL)
- 2) Testosterone (ng/mL)
- 3) Progesterone (ng/mL)
- 4) Dihydrotestosterone
- 5) Various ratios of these reproductive hormones