

HEAVY METALS, HEMATOLOGY, PLASMA CHEMISTRY, AND PARASITES IN
ADULT HELLBENDERS (*CRYPTOBRANCHUS ALLEGANIENSIS*)CHUAN-CHIN HUANG,[†] YI XU,[†] JEFFERY T. BRIGGLER,[‡] MIKE MCKEE,[§] PAUL NAM,^{||} and YUE-WERN HUANG*^{†#}[†]Department of Biological Sciences, Missouri University of Science and Technology, 105 Schrenk Hall, Rolla, Missouri 65409, USA[‡]Missouri Department of Conservation, P.O. Box 180, Jefferson City, Missouri 65120, USA[§]Missouri Department of Conservation, 1110 East College Drive, Columbia, Missouri 65201, USA^{||}Department of Chemistry, Missouri University of Science and Technology, 142 Schrenk Hall, Rolla, Missouri 65409, USA[#]Environmental Research Center, Missouri University of Science and Technology, Rolla, Missouri 65409, USA

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Abstract—Ozark (*Cryptobranchus alleganiensis bishopi*) and eastern hellbenders (*C. a. alleganiensis*) from seven rivers in Missouri, USA, were collected to investigate essential information on hematology, parasites, and plasma chemistry and levels of select heavy metals (Hg, Pb, Cd, Cr, and Co) in the animals' blood. The body masses of Ozark hellbenders were much smaller than those of eastern hellbenders. Blood parasites were detected in Ozark hellbenders, but not eastern hellbenders. The higher frequency in occurrence of eosinophils in Ozark hellbenders (8.8–16.8%) than in eastern hellbenders (highest at 6.6%) might result from the infection of parasites. Seven of the 18 hematology and plasma parameters (hematocrit, basophils, eosinophils, K, P, Ca, and chloride) showed significant differences between subspecies. The blood levels of heavy metals Co, Hg, and Pb differed significantly between subspecies. Ozark hellbenders had higher blood levels of Co ($p < 0.001$), while blood levels of Hg and Pb were higher in eastern hellbenders. The levels of chromium (Cr) and cadmium (Cd) were not different between subspecies and among rivers. The eastern hellbenders at Niangua River and the Ozark hellbenders at the North Fork of the White River had lower Hg levels compared to eastern and Ozark hellbenders at other sites. All together, our findings provide important baseline information for managing this endangered species. Environ. Toxicol. Chem. 2010;29:1132–1137. © 2010 SETAC

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INTRODUCTION

Currently, eastern (*Cryptobranchus alleganiensis alleganiensis*) and Ozark hellbenders (*C. a. bishopi*) are found in 17 states in the USA. Missouri is the only state that has both Ozark and eastern hellbenders. While both eastern and Ozark hellbenders in Missouri have experienced marked population declines, a few eastern hellbender populations in the Eastern United States are thought to be healthy and stable [1]. Data revealed a shift in age structure of hellbender populations in Missouri, with larger, mature individuals being most prevalent and young age classes being virtually absent [2,3]. Many putative threats to hellbenders exist, such as habitat degradation through increased siltation, excess nutrients, poor water quality, loss and alteration of habitat through damming and dredging, increased recreational use of their habitat, toxic chemical runoff, illegal collecting, introduced species, indiscriminant killing, and diseases and pathogens [1,4]. The Ozark hellbender is a candidate for federal listing under the Endangered Species Act with a federal recovery priority number of three. The eastern hellbender status review document has been submitted to the U.S. Fish and Wildlife Service and is currently being evaluated for federal listing [5]. Both subspecies are critically imperiled and have been listed as Missouri State Endangered Species. With the severe population decline for these two subspecies in Missouri [2,3] and the potential effects of con-

taminants in the water, more insight was needed on hematology, plasma chemistry, and burden of heavy metals in these animals.

In the past we conducted a preliminary survey on hematology and plasma chemistry in Ozark hellbenders [6] and assessed levels of organic chemicals and nutrients in the water where hellbenders are found [6]. Six of the 18 hematology and plasma chemistry parameters in hellbenders differed between rivers. Nine synthetic organic chemicals were detected. In the present study we expand our effort to include Ozark hellbenders in the Current River and eastern hellbenders inhabiting Big Piney River, Gasconade River, and Meramec River in Missouri in order to establish a broader base of knowledge on hellbender's blood parameters that pertain to immunological, excretory, regulatory, and homeostatic functions. Descriptions of these parameters correspond to the general knowledge of various amphibians and reptiles [6]. While some functions of a parameter are universal across species, other functions are species-dependent.

Recently, a necropsy report was received for a moribund Missouri hellbender sent to the National Wildlife Health Laboratory in Madison, Wisconsin, USA. As part of that necropsy, the liver was analyzed for heavy metals based on wet body weight. Levels of As, Hg, Pb, Sn, Se, and Mo were below detection limits. However, trace levels of Cd (0.435 $\mu\text{g/g}$), Co (0.868 $\mu\text{g/g}$), and Cr (0.813 $\mu\text{g/g}$) were found in the liver. Cobalt exposure increases mortality in various animals [7]. Exposure to chromium results in loss of lipid and glycogen stores as well as decreased protein content in vital organs in aquatic animals [8]. Cadmium is widely distributed in the aquatic environment and can act as an endocrine disruptor in aquatic organisms [9,10]. Cadmium can also alter growth and

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development in amphibians [11–13]. Although Pb and Hg were below the detection limits in this single hellbender tested, Pb has been found at elevated levels in surface and groundwater from certain areas in the Ozarks region in Missouri [14]. The enzymatic activity of δ -aminolevulinic acid dehydratase, a heme biosynthesis enzyme, was inhibited in fish at highly Pb-contaminated sites [15]. Furthermore, exposure to Pb resulted in a slower growth rate and spinal deformity in northern leopard frog (*Rana pipiens*) [16]. In recent years a national upward trend of Hg in the environment has been identified [17]. Dietary Hg can suppress mating behavior in fathead minnows (*Pimephales promelas*) [18]. Mercury bioaccumulation in northern two-lined salamanders (*Eurycea bislineata bislineata*), green frog (*Rana clamitans*) and bullfrog (*Rana catesbeiana*) has been reported [19,20]. Exposure to Hg caused lethality and reproductive changes in southern leopard frog larvae [21]. In general, the five major heavy metals of concern (Cr, Cd, Co, Pb, Hg) impose adverse affects on the health of aquatic organisms [22].

We had three objectives in the present study. First, we investigated essential information on hematology, parasites, and plasma chemistry in wild hellbenders throughout Missouri. Second, we determined blood levels of select heavy metals in the animals. Finally, we correlated the heavy metals data with total length and body mass to investigate heavy metal accumulation trends in short-term exposure.

MATERIALS AND METHODS

Animal and blood sample collection

Ozark and eastern hellbenders were collected from seven rivers in Missouri between May 26, 2006 and September 27, 2006. Ozark hellbenders were collected from the Current River (CR), the Eleven Point River (EPR), and the North Fork of the White River (NFR) in southern Missouri. Eastern hellbenders were collected from the Big Piney River (BPR), the Gasconade River (GR), the Meramec River (MR), and the Niangua River (NR) in south central Missouri. We intended to take 10 blood samples from each river to evaluate hematology and plasma parameters of animals within and among rivers; however, the final sample sizes varied among rivers due to relative abundance of animals. To reduce excessive blood drawing, animals from the EPR were only analyzed for heavy metals because hematology and plasma chemistry information was collected in a previous study [6].

Animals were collected by hand using the standard mask and snorkel survey technique. Upon capture, animals were scanned with an AVID Power Tracker IV to determine if they were tagged. If not tagged, animals were implanted with an AVID passive integrated transponder under the skin in the upper part of the tail. Body mass, total length, snout–vent length, gender, and morphological abnormalities were recorded. One milliliter of blood was removed for every 100 g of body weight [6]. All blood removal and processing occurred immediately after capture and was completed within a few minutes. Blood was drawn from the caudal vein slightly posterior of the cloaca. A small amount of blood was used to make two blood smears and to fill two capillary tubes. The capillary tubes were centrifuged for 1 min to separate plasma and blood cells. Both the blood smears and capillary tubes were stored in a cardboard holder at ambient temperature. Approximately 0.3 ml of whole blood was stored in liquid nitrogen and later transported to the U.S. Geological Survey Columbia Environmental Research

Center (Columbia, MO) to analyze Hg, Cr, Cd, Co, and Pb. The remaining blood was placed in heparinized vacutainers and centrifuged at 300g for 10 min. The plasma was removed and immediately placed into a liquid nitrogen dewar. Plasma samples, blood smears, and capillary tubes were analyzed for hematological analysis and plasma chemistry by Antech Diagnostics Laboratory.

Hematology and plasma chemistry analysis

The Antech Diagnostics Laboratory performed comprehensive chemistries and complete blood counts (CBC). A Hitachi 747-200 or a Hitachi 717 Chemistry Analyzer was utilized to perform the photometric assays. The protocols have been used in our previous publication [6]. The 13 parameters analyzed were glucose, urea nitrogen, total protein, albumin, aspartate aminotransferase (AST), calcium, phosphorus, globulin, creatine phosphokinase (CPK), uric acid, sodium, potassium, and chloride. For the CBCs a spun packed cell volume (Hct) was performed and then a blood smear was reviewed under a $\times 40$ lens to estimate the number of white blood cells (WBCs) and the presence of parasites. Thrombocytes were present in all hellbenders, mostly as clusters. Because of the clustering effect, no count was performed.

Heavy metal analysis

Hellbender blood samples were lyophilized in their original sampling containers as received from the field, with percent moisture being determined as part of the lyophilization process. After lyophilization, dried blood samples were mechanically ground and mixed with a plastic spatula to a fine powder. All dried samples were stored in a desiccator until time of chemical preparation.

Chemical preparation

To prepare blood samples for subsequent analysis of Cr, Co, Cd, and Pb by inductively coupled plasma mass spectrometry (ICP-MS), an aliquot of dried blood (average 37 mg) was weighed into a 10-ml Teflon[®]-lined, screw-capped borosilicate test tube, and 0.5 ml HNO₃ was added. After 1-h predigestion at room temperature the tube was sealed and placed in a hot-block heater at 110°C for 30 min. The tube was then cooled for 10 min, after which 0.2 ml high-purity H₂O₂ was added, and the tube was returned to the hot-block for 30 min. After cooling the sample was diluted to a final volume of 50 ml. Final acid matrix was 1% HNO₃.

For determining Hg in hellbender blood samples, there was no chemical preparation (digestion) because the dried sample was thermally decomposed during instrumental analysis.

Instrumental analysis

Analyses for Cr, Co, Cd, and Pb were conducted using a PE/SCIEX Elan 6000 ICP-MS, which was set up and optimized according to the manufacturer's specifications. Samples were automatically delivered to the ICP-MS by means of a software-controlled CETAC ASX-500/ADX-100 autosampler/autodiluter system. All sample digestates were analyzed with X 0 predilution by autodiluter.

The ICP-MS quantitative method was set up to determine the following masses: ⁵²Cr and ⁵³Cr, ⁵⁹Co, ¹¹¹Cd and ¹¹⁴Cd, and Pb as the sum of three masses (²⁰⁶Pb + ²⁰⁷Pb + ²⁰⁸Pb). The internal standards were Sc (10 ng/g), Rh (10 ng/g), and Bi (10 ng/g),

which were metered into the sample line via peristaltic pump. Calibration standards for analyses were 1.5, 3.0, 6.0, and 12.0 ng/ml for Cd and 5, 10, 20, and 40 ng/ml for Cr, Co, and Pb. During the actual analysis, any digestate over the concentration of the upper calibration standard for any element was automatically diluted $\times 10$ in a serial fashion until its concentration was below this level. Data were transported into Excel[®] for final compilation. Samples concentrations in $\mu\text{g/g}$ dry weight were reported for the following masses based on minimal interferences: ^{53}Cr , ^{59}Co , ^{114}Cd , and $^{206}\text{Pb} + ^{207}\text{Pb} + ^{208}\text{Pb}$.

Mercury was determined with a direct mercury analyzer (Milestone DMA 80). With this method a dried blood sample (20–25 mg) was combusted in a stream of oxygen. All Hg in the sample was volatilized and trapped by amalgamation on a gold substrate and was thermally desorbed and quantitated by atomic absorption spectrophotometry. The entire sequence was conducted with a Milestone DMA-80 analyzer equipped with an automated sample carousel. Blood mercury in this study is measured as total Hg in both inorganic and organic forms.

Statistical analysis

Prior to analysis, all datasets were tested for and met with the assumptions of parametric statistical analyses. Student's *t* test was applied to compare differences between two subspecies in all variables including morphology, hematology and plasma chemistry, and heavy metal. Subspecies differences in body mass, total length, snout–vent length were compared. Specifically, heavy metal levels in the blood may be affected by different environmental heavy metal burdens among rivers. Thus, for the heavy metals that show significant differences between subspecies, we further performed one-way analysis of variance with Duncan's posteriori comparison to detect the difference among rivers within subspecies. For the heavy metals that did not differ between subspecies, we pooled all sampling rivers together and performed analysis of variance with the Duncan's posteriori comparison. A significant correlation between Hg and body mass was observed. Therefore, body mass was used as a covariate in an analysis of covariance analysis to compare Hg levels of eastern and Ozark hellbenders among rivers. Significance level was set at $\alpha = 0.05$.

RESULTS

Morphological measurements

A total of 90 hellbenders were caught in seven rivers and their blood samples were taken from May through September 2006. All hellbenders were mature adults. Eastern hellbenders had larger body mass ($p < 0.001$), and were longer in both total length ($p < 0.001$) and snout–vent length ($p < 0.001$) than the Ozark hellbender. The body mass of eastern hellbenders ranged from 764.22 ± 40.4 g ($n = 11$) at GR to 884.4 ± 56.0 g ($n = 6$) at MR, while those of Ozark hellbenders varied between

493.6 ± 54.7 g ($n = 6$) at EPR and 520.3 ± 24.1 g at NFR (Table 1).

Blood parasite, hematology, and plasma chemistry

Blood parasites were not detected in the eastern hellbenders from BPR, GR, MR, and NR but were detected three (3/11) and 11 times (11/31) in the Ozark hellbenders at CR and NFR, respectively. Hematology and plasma chemistry are shown in Table 2. Eastern hellbenders showed higher percentages of basophils ($p = 0.002$) and higher levels of chloride ($p = 0.001$) than Ozark hellbenders. Ozark hellbenders had higher levels of hematocrit ($p < 0.001$), eosinophils ($p < 0.001$), potassium ($p = 0.016$), calcium ($p < 0.001$), and phosphorus ($p < 0.001$) than eastern hellbenders.

Heavy metal levels in whole blood

The levels of Cr (0.13–6.87 $\mu\text{g/g}$), Co (0.07–0.41 $\mu\text{g/g}$), Cd (0.002–0.11 $\mu\text{g/g}$), Pb (0.013–0.180 $\mu\text{g/g}$), and Hg (0.08–0.65 $\mu\text{g/g}$) were determined using lyophilized whole blood samples. Mercury had a linear correlation with total length (Fig. 1; $p = 0.005$) and body mass ($p = 0.024$; data not shown), thus total length was included as a covariate the analysis of covariance analysis of Hg levels between eastern and Ozark hellbenders.

Ozark hellbenders showed a higher burden in Co ($p < 0.001$), while eastern hellbenders had higher levels in Hg ($p = 0.0189$) and Pb ($p = 0.005$). The levels of Cr and Cd did not differ between subspecies and among all rivers. The NR and NFR showed lower Hg levels within eastern hellbenders and Ozark hellbenders, respectively (Table 3).

DISCUSSION

Data in the present study indicate that Ozark hellbenders have a smaller total body length and significantly less mass compared to eastern hellbenders. The findings corroborate previous reports that Ozark hellbenders are smaller than eastern hellbenders [23,24].

Previous studies indicated that parasites in the blood system seemed to be common in salamanders and amphibians [8,23,25,26]. In this study, none of the eastern hellbenders tested positive for blood parasites, whereas approximately 33% of the Ozark hellbenders tested positive. Eosinophils are typically elevated during helminth, protozoal, and metazoan infections [6]. Thus, the higher eosinophils occurrence in Ozark hellbenders (8.8% in NFR and 16.8% in CR) versus eastern hellbenders (highest in BPR, 6.6%) might be related to the higher incidence of blood parasites in Ozark hellbenders (Table 2). Leeches have been known to contribute to pathogen transmission in salamanders [27]. *Desserobdella cryptobranchii* (an aquatic leech) was found in Ozark hellbenders [28], but not in eastern hellbenders in Missouri (J. Briggler, personal

Table 1. Body mass, total length and snout-vent length of hellbenders

Subspecies	Eastern hellbender				Ozark hellbender		
	BPR (11)	GR (11)	MR (6)	NR (14)	NFR (31)	CR (11)	EPR (6)
Body mass	806.2 ± 40.4	764.2 ± 40.4	884.4 ± 56.0	813.3 ± 34.6	520.3 ± 24.1	496.1 ± 40.4	493.6 ± 54.7
Total length	48.8 ± 1.1	47.8 ± 1.1	50.4 ± 1.5	47.2 ± 1.0	42.0 ± 0.7	42.8 ± 1.1	42.1 ± 1.5
Snout-vent length	32.7 ± 0.9	32.2 ± 0.9	33.8 ± 1.1	32.2 ± 0.7	29.1 ± 0.5	29.4 ± 0.9	28.4 ± 1.2

Values are given as mean \pm SD. Numbers in parentheses are sample sizes. Big Piney River (BPR), Gasconade River (GR), Meramec River (MR), and Niangua River (NR) (MO, USA) are rivers with eastern h hellbenders. Current River (CR), Eleven Point River (EPR), and North Fork of the White River (NFR) are rivers with Ozark hellbenders. Statistics are described under *Morphological measurement* in the *Results* section.

Table 2. Hematology and plasma chemistry of hellbenders

Parameter	BPR (10)	GR (11)	MR (5-6)	NR (11)	NFR (31)	CR (11)
Hematocrit (%)	37.1 ± 2.3	33.6 ± 2.2	39.8 ± 2.9	36.0 ± 2.2	43.5 ± 1.3	46.7 ± 2.2
WBC estimate (10 ³ /μl)	3.9 ± 0.6	4.3 ± 0.6	3.54 ± 0.9	3.7 ± 0.6	5.1 ± 0.4	3.1 ± 0.6
Het/Poly (%)	30.4 ± 4.5	26.5 ± 4.3	13.4 ± 6.3	42.4 ± 4.5	35.0 ± 2.5	35.5 ± 4.3
Lymphocytes (%)	47.4 ± 5.5	60.4 ± 5.3	78.4 ± 7.8	43.6 ± 5.5	51.5 ± 3.2	43.0 ± 5.3
Monocytes (%)	0.3 ± 0.6	2.2 ± 0.5	1.4 ± 0.8	0.7 ± 0.6	0.5 ± 0.3	0.9 ± 0.5
Eosinophils (%)	6.6 ± 1.6	1.5 ± 1.5	0.2 ± 2.2	6.5 ± 1.6	8.8 ± 0.9	16.8 ± 1.5
Basophils (%)	5.3 ± 1.3	8.5 ± 1.2	6.6 ± 1.8	6.8 ± 1.3	4.5 ± 0.7	3.8 ± 1.2
Glucose (mg/dl)	22.2 ± 5.8	22.5 ± 5.5	11.3 ± 7.5	42.7 ± 5.5	31.4 ± 3.3	23.5 ± 5.5
Urea nitrogen (mg/dl)	3.8 ± 0.9	2.0 ± 0.9	1.8 ± 1.2	2.0 ± 0.9	3.4 ± 0.5	1.8 ± 0.9
Total protein (g/dl)	3.3 ± 0.2	2.7 ± 0.1	2.9 ± 0.2	3.7 ± 0.1	3.2 ± 0.1	3.4 ± 0.1
Albumin (g/dl)	1.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Globulin (g/dl)	2.1 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.5 ± 0.1	2.2 ± 0.1	2.3 ± 0.1
AST (SGOT) (U/L)	157.6 ± 22.6	123.2 ± 21.5	80.8 ± 29.1	130.0 ± 21.5	157.4 ± 12.8	118.5 ± 21.5
Calcium (mg/dl)	9.8 ± 0.8	8.8 ± 0.8	7.7 ± 1.0	10.0 ± 0.8	12.1 ± 0.5	12.2 ± 0.8
Phosphorus (mg/dl)	5.2 ± 0.5	4.3 ± 0.5	4.2 ± 0.6	5.7 ± 0.5	6.8 ± 0.3	7.2 ± 0.4
Sodium (mEq/L)	107.1 ± 1.2	103.8 ± 1.1	102.8 ± 1.5	110.6 ± 1.1	107.0 ± 0.7	104.4 ± 1.1
Potassium (mEq/L)	4.2 ± 0.4	4.8 ± 0.4	4.8 ± 0.5	3.4 ± 0.4	5.0 ± 0.2	5.2 ± 0.4
Chloride (mEq/L)	83.1 ± 1.4	84.5 ± 1.3	82.5 ± 1.7	86.0 ± 1.3	81.2 ± 0.8	79.8 ± 1.3
CPK (U/L)	8278 ± 1592	1261 ± 1518	2536 ± 2055	2568 ± 1518	982 ± 904	943 ± 1518
Uric acid (mg/dl)	0.3 ± 0.07	0.3 ± 0.09	0.3 ± 0.07	0.5 ± 0.04	0.4 ± 0.05	0.4 ± 0.07

BPR, GR, MR, and NR are rivers with eastern hellbenders. CR and NFR are rivers with Ozark hellbenders. Values are given as mean ± SD. Numbers in parentheses are sample sizes. Refer to Table 1 for river abbreviations.

WBC, white blood cell; AST (SGOT), aspartate aminotransferase; CPK, creatine phosphokinase.

communication). Thus, the source and spread of the blood parasite may be associated with the occurrence of *D. cryptobranchii* in Ozark hellbenders. It remains to be investigated whether parasites affect hellbender's health and consequently contribute to their decline.

Functional descriptions of hematology and plasma chemistry in various amphibian and reptilian species can be found in our previous study [13]. Lymphocytes, monocytes, eosinophils, and basophils are responsive to pathogenic infections. Although their levels could be transient, they are an effective tool for preliminarily screening disease-related conditions in hellbenders [29–31] (D.P. Jerrett, 1971, Master's thesis, Depauw University, Greencastle, IN, USA). The levels of glucose and total protein, indicators of an animal's nutritional status, varied among six rivers, indicating that they might experience differential nutritional conditions, either temporary or long-term. The glucose levels of the animals in MR were 50% or higher

compared to other rivers. Causes of the difference and potential effects on animal's development and reproduction are unknown. Creatine phosphokinase is used to diagnose and monitor liver, kidney, and heart disease in amphibians, although it can be elevated during stress. Whether the difference in CPK among rivers is a consequence of functional variations or due to stress remains to be elucidated. In 2003 higher numbers of lymphocytes ($p=0.036$) and lower levels of calcium ($p=0.001$) were observed in CR, compared to 2006. Whether animals were experiencing a better disease-related status in 2006 requires additional evidence.

The blood levels of heavy metals Co, Hg, and Pb significantly differ between the two subspecies. River-specific exposure and differences in disposition of these two subspecies remain to be investigated. The sources of heavy metals in hellbenders may come from their main dietary item, crayfish, whose four heavy metal levels ranged from 0.14 μg/g to 3.06 μg/g whole body dry weight [32].

Blood Hg differed among rivers within the subspecies. The Ozark hellbenders at NFR and the eastern hellbenders at NR had the lowest average concentrations. The blood Hg in Ozark hellbenders at CR and in eastern hellbenders at BPR was approximately three times higher than those at NFR and NR, respectively.

Blood Hg positively correlates with total length and body mass, indicating accumulation in short-term exposure. Diet may be the major source of accumulated Hg [21,32]. Similar correlations were also observed in fish [33]. Accumulation of heavy metals and their toxic effects have been reviewed in various species [34–36]. Studies on blood Hg levels in fish can be used to gauge the uniqueness of hellbender Hg levels. Brumbaugh et al. [37] reported blood levels of Hg ranging from 0.6 to 81.2 nL/L wet weight in smallmouth bass (*Micropterus dolomieu*) from streams with very similar river characteristics to ours. After unit conversion using moisture contents in hellbender blood samples, the maximal blood Hg in hellbenders (85.8 nL/L) was nearly identical to those observed in smallmouth bass (81.2 nL/L wet wt). The mean Pb levels in hellbender whole blood differed between subspecies. Within the subspecies there

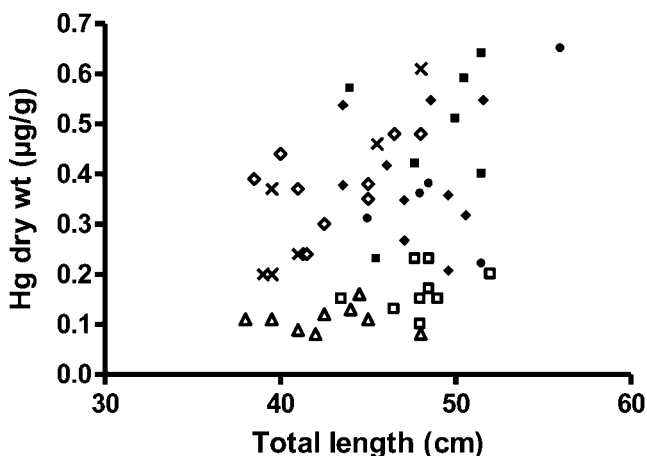


Fig. 1. The total length – Hg concentration dispersal graph showed the trend of correlation between mercury levels and total length of hellbenders in the Big Piney River (BPR; ■), Current River (CR; ◇), Eleven Point Point (EPR; ×), Gasconade River (GR; ◆), Meramec River (MR; ●), Niangua River (NR; □), and the North Fork of the White River (NFR; △) (MO, USA).

Table 3. Levels of five heavy metals in hellbenders (mean \pm SD)

Metal	BPR (4–7) ^a	GR (5–10)	MR (2–5)	NR (8–10)	NFR (8–9)	CR (9)	EPR (4–6)
Hg ^b	0.48 \pm 0.14A ^c (0.26–0.64) ^d	0.40 \pm 0.12A	0.38 \pm 0.16A	0.17 \pm 0.04B	0.11 \pm 0.02A	0.38 \pm 0.08A	0.35 \pm 0.17B
Pb	0.077 \pm 0.029 (0.042–0.110)	0.088 \pm 0.056 (0.029–0.180)	0.075 \pm 0.025 (0.033–0.096)	0.088 \pm 0.064 (0.024–0.150)	0.044 \pm 0.013 (0.031–0.057)	0.055 \pm 0.049 (0.013–0.180)	0.046 \pm 0.01 (0.026–0.056)
Co	0.29 \pm 0.06 (0.24–0.31)	0.39 \pm 0.26 (0.16–0.85)	0.29 \pm 0.13 (0.07–0.38)	0.48 \pm 0.25 (0.19–0.96)	0.95 \pm 0.31 (0.84–1.41)	0.85 \pm 0.29 (0.53–1.32)	0.74 \pm 0.23 (0.53–1.15)
Cr	0.73 \pm 0.29 (0.47–1.11)	0.88 \pm 0.53 (0.48–2.16)	0.49 \pm 0.52 (0.13–1.3)	2.02 \pm 1.82 (0.52–6.87)	0.92 \pm 0.46 (0.58–1.69)	1.18 \pm 0.58 (0.34–1.91)	0.66 \pm 0.27 (0.47–1.04)
Cd	0.006 \pm 0.005 (0.002–0.01)	0.002 \pm 0.000 (0.002–0.002)	0.002 \pm 0.000 (0.002–0.002)	0.018 \pm 0.038 (0.002–0.11)	0.022 \pm 0.007 (0.011–0.032)	0.020 \pm 0.016 (0.007–0.057)	0.016 \pm 0.007 (0.007–0.028)

The metal level in hellbenders is $\mu\text{g/g}$ dry whole blood weight. BPR, GR, MR, and NR are rivers with eastern hellbenders while CR, EPR, and NFR are rivers with Ozark hellbenders. Refer to Table 1 for river abbreviations.

^a Numbers in parentheses are sample size.

^b Significant difference between eastern and Ozark hellbenders.

^c Capitalized letters within the same row indicate the significant differences at $\alpha = 0.05$ among rivers within subspecies.

^d Values in parentheses are the ranges of heavy metal levels.

were no differences among rivers. Biotic and abiotic factors attributing to the differences as well as sensitivity of hellbenders to Pb toxicity remain to be investigated.

The Cr levels in the blood did not differ between subspecies and among rivers. The blood levels of Cd in animals were not different between subspecies and among sampling sites, with the measurements being quite low or near the limit of detection 0.002 $\mu\text{g/g}$.

The present study contributes several important findings in biology and conservation of hellbenders. First, blood parasites were not detected in the eastern hellbenders, while blood parasites were detected with high frequency in the Ozark hellbenders in all rivers investigated. Second, most of the hematology and plasma chemistry varied between subspecies in Missouri. Third, Hg and Pb are accumulated in both eastern and Ozark hellbenders. The source of these accumulated heavy metals may be from the major diet, crayfish. How parasites and selective heavy metals influence subspecies-specific physiological conditions as well as acting as possible causes of population decline deserves further attention. Further, with enough data accumulated in the future, we intend to understand relationships between blood chemistry and heavy metals. Collectively, our findings provide important baseline information for managing this endangered species.

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