Herpetological Conservation and Biology 5(1):44-48.
Submitted: 13 January 2009, Accepted: 11 January 2010.

SEXUAL DIMORPHISM IN THE EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*)

ROBERT MAKOWSKY[1,3], LAWRENCE A. WILSON[2], AND THOMAS K. PAULEY[1]

[1]Department of Biological Sciences, Marshall University, One John Marshall Drive, Huntington, West Virginia 25755, USA
[2]Fernbank Science Center, 156 Heaton Park Drive N.E., Atlanta, Georgia 30307, USA
[3]Present Address: Department of Biostatistics, University of Alabama at Birmingham, 327 Ryals Public Health Building, Birmingham, Alabama 35294, USA, email: makowsky@uab.edu

***Abstract.*—Sexual dimorphism is common in vertebrates that exhibit male-male combat. The Eastern Hellbender, *Cryptobranchus a. alleganiensis*, exhibits such combat and sexual dimorphism has been reported. The most easily discernable dimorphic character, swelling around the cloaca in males, is detectable only during and around the autumn breeding season. We used univariate and multivariate analyses to determine if other morphological characters could be used to determine the sex of adult hellbenders outside the breeding season. We analyzed six morphological features of 105 preserved specimens and found that males differed significantly from females in snout-vent length, thoracic girth, and mass. However, the differences detected were not sufficient to allow positive determination of sex of an individual. Although hellbenders exhibit sexual dimorphism in several morphological characters, the only reliable external morphological character is the presence of swelling around the cloaca of males during the autumn breeding season.**

Key Words.—Cryptobranchus; Hellbender; morphology; sexual dimorphism

INTRODUCTION

The Hellbender, *Cryptobranchus alleganiensis*, is one of North America's largest salamanders (Petranka 1998). Unfortunately, like many other amphibians (Stuart et al. 2004), it is a species in decline (Williams et al. 1981; Bother and Gottlieb 1991; Wheeler et al. 2003; Bauman and Wilson 2005; Nickerson and Briggler 2007). Captive breeding attempts have been, thus far, unsuccessful. Unless obstacles hindering captive breeding can be overcome, conservation of hellbenders will be dependent on management of wild populations. Detailed demographic information is imperative to ensure that management efforts are successful. Natural history and demography of hellbenders have been studied at several localities (e.g., Taber et al. 1975; Bother and Gottlieb 1991; Humphries and Pauley 2005). Sex ratios have been included with demographic data, but such data have been limited to the breeding season when males can be reliably distinguished from females. Limited data exists that allows for determination of the sex of hellbenders outside of the breeding season.

Sex of hellbenders is readily determined during the breeding season when the area around the cloaca of males swells (Nickerson and Mays 1973). Ultrasound images and serum calcium analyses could be useful for determining sex, but such methods are either not practical (ultrasound) or have not been thoroughly evaluated for their ability to determine sex (serum calcium). Additional dimorphisms that have been reported are subjective, difficult to reproduce, or possibly population specific (King 1939; Bishop 1941). Currently, no morphological feature or suite of features is known that will allow for the determination of sex outside the breeding season or for immature specimens. Two previous studies on populations in Missouri (Taber et al. 1975) and West Virginia (Humphries and Pauley, 2005) have reported that mature females are heavier than mature males when size corrected. Conversely, because male-male combat has been reported (Alexander 1927), it is expected that males will be larger than females (Shine 1979; Shine 1989), either overall (i.e., longer total length) or when size corrected (i.e., length-corrected mass). Even though theoretical reasoning and empirical data suggest that a dimorphism should exist, it has not been determined if such differences can be quantified and used to predict sex. We sought to determine if sexually dimorphic characters could be used to reliably predict sex in a quantitative framework throughout the year. This ability would improve population monitoring efforts by providing researchers with an objective and readily available sex-determining method. This capability is necessary because, without an accurate estimate of effective population size, it is impossible to determine the status of a population and predict with any certainty about future trends.

METHODS

We measured 15 characters of 105 preserved Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) stored in 70% ethanol at either the Carnegie Museum of Natural History or the West Virginia Biological Survey Museum at Marshall University. Total length ranged from 17.5–

57.9 cm for males (n = 53) and from 36.4–56.3 cm for females (n = 52). All hellbenders were collected from populations in Pennsylvania (n = 63), West Virginia (n = 14), and Virginia (n = 28).

We divided measurements into two categories: "primary" measurements, those that are routinely measured in the field, and "secondary" measurements, those that are difficult to obtain accurately and precisely without anesthetizing or euthanizing the specimen, but are important dimorphic characteristics in other caudates (Shine 1979). Primary measurements included snout-vent length (SVL), tail length (TAIL), total length (TL), head width (HW), cube root mass ($MA^{1/3}$), and thoracic girth (TG). Secondary measurements included head height, eye-to-eye distance, eye-to-nostril distance, eye-to-cross-nostril distance, nostril-to-nostril distance, tail height, first-toe length, third-toe length, and axilla-to-groin length. We determined $MA^{1/3}$ with a triple beam balance, thoracic girth (just posterior to the front limbs) with a string that we measured while we stretched it on a ruler, and all other measurements with digital calipers (Ohaus Corp., Pine Brook, New Jersey, USA). We recorded all measurements three times and one measurement of the three was randomly chosen for all analyses. To avoid flawed measurements, we took care not to depress the flesh to a point that would displace tissue. We subsequently determined sex by dissecting individuals and examining the gonads. Student's t-tests were done in Minitab 14 (Minitab Inc., State College, Pennsylvania, USA) unless the test's assumptions were violated (see Table 1). We calculated size-corrected measurements by dividing HW, TG, $MA^{1/3}$, SVL, and TAIL by SVL and/or TL and using the arcsine square-root transformation (Table 1). We employed principal components analysis (PCA) and discriminate function analysis (DFA) with SAS (SAS Institute Inc., Cary, North Carolina, USA) using the primary raw measurements and sex as the grouping variable. Unfortunately, detailed information concerning the specific date of death and whether specimens were held in captivity was not available for most specimens, so we did not include date as a covariate.

We initially employed a comparison using all 15 measurements (both primary and secondary). Because most of the secondary measurements are difficult to obtain from live, non-anesthetized hellbenders, their usefulness in determining sex was initially tested on a subset of the specimens (West Virginia sample). None of the secondary measurements approached significance (all $P >> 0.1$); consequently, they were not included in additional analyses.

Shrinkage commonly occurs in salamanders following fixation and preservation. To determine if the amount of shrinkage differed between males and females, we compared pre- and post-preservation TL, SVL and $MA^{1/3}$ of 10 hellbenders (n = 6 females, 4 males) collected from a single population in Virginia on June 3, 1984. These hellbenders were deposited by the same person and stored in 70% ETOH, but no information was recorded concerning fixation method.

RESULTS

We found significant sexual dimorphism associated with three of the primary measurements (Table 1). However, the amount of overlap between sexes for each of these characters was too large for them to be useful in determining the sex of an individual hellbender. Despite the presence of male-male combat predicting that males attain a larger overall size, neither comparisons of TL, SVL, nor $MA^{1/3}$ supported such a conclusion. Therefore, we focused on length-correcting ratios to determine if sexual dimorphism was present in specimens.

Only one ratio was found to differ significantly between sexes: thoracic girth/total length (Mann-Whitney, df = 103, $P = 0.048$; Table 1). However, the large overlap in value between sexes was too great to accurately determine sex of an individual. Principal components analysis showed a large overlap between the sexes on principal components 1 and 2 (Fig. 1). DFA was able to predict sex only 72% of the time for males and 65% of the time for females.

The TL and SVL were significantly shorter and $MA^{1/3}$ significantly less in all specimens following 25 years of preservation, but the shrinkage associated with preservation was not significantly different between males and females (Table 2). Overall, length was reduced by 5.9%; whereas, $MA^{1/3}$ was reduced by 12.6%.

DISCUSSION

Although we detected significant dimorphism, we found no definitive means to distinguish sex of Eastern Hellbenders using these measurements. Therefore, although some of the measurements and ratios were different between sexes, overlap in these characters between the sexes indicates that within-sex variation is almost as great as between-sex variation. Our analysis of pre- and post-preservation characters (i.e. no difference in changes associated with preservation affects males and females equally) also leads us to conclude that live specimens would exhibit a similar level of dimorphism. One limitation of this study is the lack of data when the hellbenders were collected and killed. If hellbenders were held in captivity for weeks or months, then two problems may arise. First, the collection date may not coincide with the preservation date, which was not recorded for most of specimens in this study. Second, hellbenders in captivity experience different environmental conditions than those in the wild (e.g., availability of food, stress, temperature). Such

TABLE 1. Comparison of male (N = 53) and female (N = 52) Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) for each measurement and ratio. All tests have 103 degrees of freedom. t = Student's t-test, W = Mann-Whitney Rank Sum Test. Measurement variables are: TL = total length, SVL = snout-vent length, HW = head width, TG = thoracic girth, $MA^{1/3}$ = cube root mass, and TAIL = tail length.

Measurement or Ratio	Male mean	Std. Error	Female mean	Std. Error	Test statistic	*P* value
TL	42.5	1.18	45.5	0.71	W = 3007	0.108
SVL	27.7	0.72	30.1	0.48	W = 3098	0.029
HW	5.5	0.17	5.9	0.11	W = 2957	0.199
TG	16.5	0.44	18.46	0.38	t = -3.23	0.001
$MA^{1/3}$	7.3	0.19	8.0	0.14	t = -2.95	0.004
TAIL	14.7	0.52	15.4	0.29	W = 2954	0.204
TG/ TL	0.39	0.0056	0.41	0.0055	t = 2.01	0.048
TG/ SVL	0.60	0.0088	0.61	0.0090	t = -1.46	0.148
$MA^{1/3}$ / TL	0.17	0.0019	0.18	0.0017	t = -1.15	0.254
$MA^{1/3}$ / SVL	0.27	0.0029	0.27	0.0031	t = -0.48	0.630
HW/ TL	0.129	0.0016	0.13	0.0014	t = -0.29	0.770
HW/ SVL	0.12	0.0026	0.20	0.0022	t = 0.26	0.793
TAIL/ TL	0.34	0.0043	0.34	0.0032	W = 4736	0.336
SVL/ TL	0.66	0.0043	0.66	0.0032	W = 2860	0.509

differences in environmental conditions could affect growth and, consequently, our measurements. Because we have no way to determine if the hellbenders we measured were held in captivity or killed immediately, we were unable to incorporate date of collecting into our analyses.

Because hellbenders are known to engage in male-male combat during the breeding season (Smith 1907)

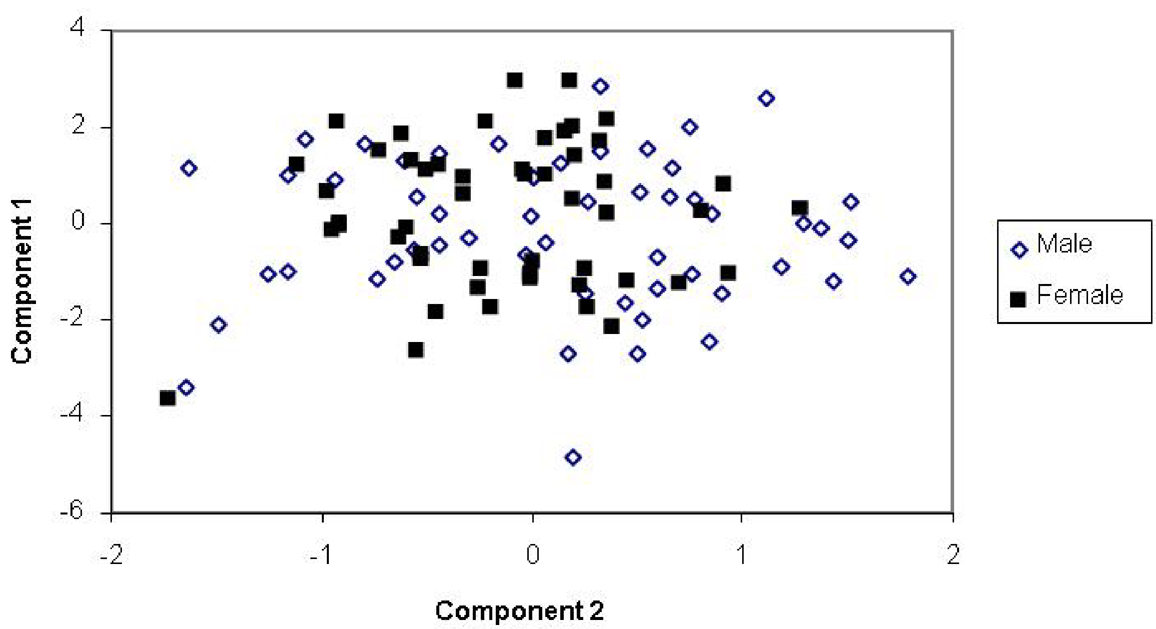

FIGURE 1. Separation of males and female Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) using principal components analysis on raw measurements. Notice the high degree of overlap between sexes.

TABLE 2. Percentage of shrinkage in Eastern Hellbender (*Cryptobranchus a. alleganiensis*) specimens after being preserved for 20 years, and the significance level for both sexes combined. Abbreviations: SVL = snout-vent length, TL = tail length, *t* = Student's t-test, W = Whitney Rank Sum test, and df = degrees of freedom.

Measurement	Mean shrinkage of females(n = 6)	Mean shrinkage of Males (n = 4)	Mean of all specimens, df = 9	Difference between sexes, df = 8
$MA^{1/3}$	12.7%	13.7%	13.1% $t = 51.6$, $P < 0.001$	1% $t = 0.31$, $P = 0.766$
SVL	6.2%	4.5%	5.5% $t = 100.4$, $P < 0.001$	1.7% W = 29, $P = 0.455$
TL	6.9%	4.1%	5.6% $t = 54.3$, $P < 0.001$	2.8% $t = -0.80$, $P = 0.448$

and exhibit at least one sexually dimorphic morphological character (swelling around the cloaca in males), we were surprised at our inability to detect sexual dimorphism in other morphological features. We also did not find that females are significantly heavier than males of the same length like previous studies (Taber et al. 1975; Humphries and Pauley 2005). These previous studies focused on specimens captured during the breeding season, though, and it would be expected for females to be heavier due to the presence of eggs. Therefore, our results suggest that females are not significantly heavier than males sampled outside of the breeding season. We did find that females have a larger girth/SVL ratio than males. Based on the lack of difference in size-corrected mass observed in males and females, the relatively larger girth of females is most likely not attributable only to eggs in females (most pronounced at the beginning of breeding season). Although males and females do not differ in overall size attained based on the specimens studied, this does not imply such differences do not occur. In this study, the largest specimen was a male with a total length of 58 cm, 16 cm shorter than the largest confirmed record (Fitch 1947). Therefore, it is possible that overall size can be useful in determining sex for specimens at sizes larger than what we examined. Due to the paucity of large preserved specimens as well as our experience in rarely finding specimens over 60 cm in nature (Bowles and Wilson 2000; Makowsky and Wilson 2001; Humphries and Pauley 2005), overall size, and morphological dimorphism in general, are probably not helpful sex determining characters.

In this study we sought to determine if sexually dimorphic characters could be used to reliably predict sex in a quantitative framework so that researchers would have an inexpensive, widely available and objective method at their disposal. We found no evidence that the characters we examined are useful for such a purpose. The need to definitively determine sex outside of the breeding season still exists, though, and without such a method demographic studies will continue to suffer. Interestingly, serum calcium level has been shown to differ between sexes (Solis et al. 2007), but its usefulness in determining sex has not been assessed. This method should be examined further, as it may provide a practical way to ascertain sex throughout the year.

Acknowledgments.—We would like to thank the Carnegie Museum of Natural History for loaning many of the specimens used in this study. John Morse, Brian Fontenot, Kris Cox, Leslie Rissler, and Jeff Humphries provided helpful comments on previous versions of the manuscript. Funding was graciously provided by the West Virginia Department of Natural Resources and the Marshall University Graduate School.

LITERATURE CITED

Alexander, W.P. 1927. The Allegheny hellbender and its habitat. Buffalo Society of Natural Sciences (Hobbies) 7:13–18.

Bauman, M L., and L.A. Wilson. 2005. The population status and limiting factors of the Eastern Hellbender in the Tennessee River drainage of Georgia. Southeastern Biologist 52:153.

Bishop, S.C. 1941. Salamanders of New York. New York State Museum Bulletin 324:1–365.

Bother, R.C., and J.A. Gottlieb. 1991. A study of the New York State populations of the hellbender *Cryptobranchus alleganiensis alleganiensis* (Daudin). Proceedings of the Rochester Academy of Science 17:41–54.

Bowles, R., L.A. Wilson. 2000. Population study of the Hellbender in southeastern United States. Association of Southeastern Biologists Bulletin 47:163.

Fitch, F.W. 1947. A record of *Cryptobranchus alleganiensis*. Copeia 1947:210.

Humphries, W.J., and T.K. Pauley. 2005. Life history of the Hellbender, *Cryptobranchus alleganiensis,* in a

West Virginia stream. American Midland Naturalist 154:135–142.

King, W. 1939. A survey of the herpetology of the Great Smoky Mountain National Park. American Midland Naturalist 21:531–582.

Makowsky, R., L.A. Wilson. 2001. Diet of *Cryptobranchus alleganiensis* in the southern Appalachians. Southeastern Biologist 48:156.

Nickerson, M.A., and J.T. Briggler. 2007. Harvesting as a factor in population decline of a long-lived salamander; the Ozark Hellbender, *Cryptobranchus alleganiensis bishopi*. Applied Herpetology 4:207–216.

Nickerson, M.A., and C.E. Mays. 1973. The Hellbenders. Milwaukee Public Museum, Milwaukee, Wisconsin, USA.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, D.C., USA.

Shine, R. 1979. Sexual selection and sexual dimorphism in the Amphibia. Copeia 1979:297–306.

Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: A review of the evidence. The Quarterly Review of Biology 64:419–455.

Smith, B.G. 1907. The life history and habits of *Cryptobranchus allegheniensis*. Biological Bulletin 13:5–39.

Solis, M.E., J.M. Bandeff, and Y. Huang. 2007. Hematology and serum chemistry of Ozark and Eastern Hellbenders (*Cryptobranchus alleganiensis*). Herpetologica 63:285–292.

Stuart, S.N., J.S. Chanson, N.A Cox, B.E. Young, A.S.L. Rodrigues, D.L. Fischman, and R.L. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1883–1885.

Taber, C.A., R.F. Wilkinson, Jr., and M.S. Topping. 1975. Age and growth of hellbenders in the Nianuga River, Missouri. Copeia 4:633–639.

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 109:151–156.

Williams, R.D., J.E. Gates, C.H. Hocutt, and G.J. Taylor. 1981. The Hellbender: A nongame species in need of management. Wildlife Society Bulletin 9:94–100.

Robert Makowsky is currently a Post-Doctoral Fellow in the Department of Biostatistics at the University of Alabama at Birmingham. He received a B.S. degree from Emory University, a M.S. from Marshall University, and a Ph.D. from the University of Texas at Arlington. His general interests include phylogenetics, population genetics, and information-based inference. (Photographed by anonymous)

Lawrence A. Wilson has been an ecologist at Fernbank Science Center for over 25 years as well as an adjunct professor in the Environmental Studies Department at Emory University in Atlanta. He has been working on hellbenders for over twenty years with other areas of study being tropical ecology, plant secondary chemistry, and herpetological conservation. (Photographed by Virginia Dunbar)

Thomas K. Pauley has taught and worked with college students since 1966. He is currently a professor at Marshall University where he has mentored over 75 graduate students. He received a B.S. degree from the University of Charleston, a M.S. from Marshall University, and a Ph.D. from West Virginia University. His research interests include conservation and natural history of amphibians and reptiles in the central Appalachians. (Photographed by Mark Watson)