



Hellbender (*Cryptobranchus alleganiensis*) populations are in decline.

Saving a Giant Salamander

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The largest salamander in North America is in decline (Wheeler et al. 2003). Hellbenders (*Cryptobranchus alleganiensis*) are the only representative of the family Cryptobranchidae that occurs outside of Asia, and are second in size only to their fellow cryptobranchids, the Asian Giant Salamanders (genus *Andrias*). In addition to being large, these salamanders are unusual in that they inhabit rivers where they co-exist with numerous fish species. Hellbenders have strict habitat requirements, including cool, fast-flowing waters with rocky substrates. Individuals are cryptic, with a mottled gray/brown coloration, and are dorsoventrally flattened to fit under large rocks (Petranka 1998). Because they depend heavily on cutaneous respiration, their bodies are covered with wrinkled skin that maximizes the surface area for gas absorption. Juveniles remain as

gilled larvae for 2–3 years and do not reach sexual maturity for 5–6 years (see Nickerson and Mays 1973 for an overview).

Because of their unusual appearance and life history, Hellbenders have received considerable attention from researchers, including intensive monitoring of some populations during the last half century. Two apparently paraphyletic subspecies of the Hellbender are currently recognized (Sabatino and Routman 2008). The eastern subspecies (*C. a. alleganiensis*) is distributed throughout the Appalachian region, reaching as far west as Missouri, and the Ozark subspecies (*C. a. bishopi*) is only found in a relatively small area of southern Missouri and northern Arkansas (Petranka 1998). In some eastern regions, Hellbender populations appear relatively healthy (Humphries and Pauley 2005, Nickerson et al. 2002), whereas declines in other populations, especially in the western portion of the species' range, have been quite large. In five Missouri streams, Hellbender population sizes have declined by over 75% since the 1970s (Wheeler et al. 2003). These sharp declines are characterized by age distributions that reveal a disproportionate decrease in numbers of smaller (younger) individuals. The Spring River in Arkansas once was home to robust numbers of Ozark Hellbenders (370 individuals marked in one study; Peterson 1985), but an intensive survey in 1991 yielded only 20 individuals. The Eastern Hellbender is now considered to be state-endangered throughout much of its range. Because all populations in the narrow range of the Ozark Hellbender are experiencing declines, this subspecies is currently a candidate for federal listing as endangered.

As is the case for many threatened species, the cause of population declines for Hellbenders is not known. However, detailed lists of possible contributing factors have been compiled (Mayasich et al. 2003; IUCN/SSC 2006). These factors include biological threats (disease and competition/predation by exotic species), overcollection for research, teaching, or commercial use, water quality issues (silt-



Hellbenders occupy cold, fast-flowing streams with high concentrations of dissolved oxygen.



Lateral skin folds help Hellbenders respire by increasing the surface area for gas exchange through the skin.



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The diet of Hellbenders consists primarily of crayfish.

tion, impoundments, agricultural runoff, runoff from urban/rural development, waste products from humans), and climate change. Progress is being made in investigating some of these issues.

Disease

One pathogenic fungus, *Batrachochytrium dendrobatidis* or 'chytrid,' is known to be responsible for amphibian declines throughout the world (e.g., Skerratt et al. 2007). Recently, the chytrid fungus has been found on Hellbenders in five different rivers in Missouri and Arkansas (Briggler et al. 2008), and up to 25% of Hellbenders that have been tested from populations in Missouri have tested positive (Briggler 2007). The symptoms of chytrid infection (Berger et al. 1998) are similar to the health problems (see below) found in some Hellbenders, but so far the number of infected adults has not been adequate to look for a correlation between chytrid infections and these problems (J. Briggler, pers. comm.). Regardless, at the very least, the chytrid fungus probably works as a stressor to exacerbate problems from other sources (J. Briggler, pers. comm.).

Physical Abnormalities

A particularly alarming finding in declining populations is that many of the remaining Hellbenders have mild-to-severe physical abnormalities. Interpretation of these problems is complicated because Hellbenders are aggressive during the breeding season, and wounds from bites from other Hellbenders are common at those times

(Humphries and Pauley 2005, Miller and Miller 2005). However, researchers who have studied these populations for many years have concluded that the number and severity of abnormalities have increased dramatically over the last few decades (Hiler et al. 2005; M. Nickerson, R. Wilkinson, S. Trauth, pers. comm.). Physical abnormalities were exhibited by 41% of adults (and no juveniles) in Tennessee and by 25% of individuals in Ohio. In Arkansas and



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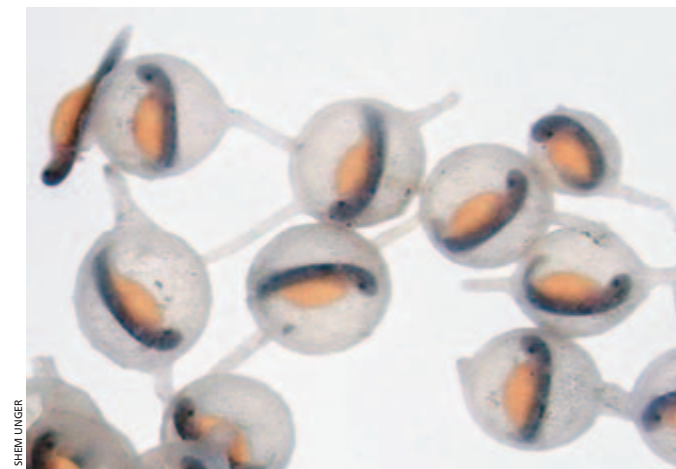
Abnormal digit sores found on a wild-caught adult Hellbender.

Missouri, the number of abnormalities varies among populations. Hiler et al. (2005) reported that 90% of the small remaining population of Ozark Hellbenders in the Spring River and 40% in the Eleven Point River in Arkansas exhibited abnormalities, and Briggler (2007) stated that abnormalities were more common for the Ozark subspecies than the eastern subspecies in Missouri. In addition to bite marks from other Hellbenders, Hiler et al. (2005) categorized the abnormalities as tumors, open wounds, fungal infections, necrotic limbs, missing limbs, digital abnormalities, eye abnormalities, and cloacal wounds. The causes of the dramatic increases in abnormalities are not yet known.

Predation by Exotic Species (Trout)

One hypothesis for the current low recruitment observed in some Hellbender populations is increased predation on larvae and/or eggs. Hellbenders are likely subject to predation from a variety of predators, including native fishes (e.g., bass, sculpin, walleye), but without evidence of growth in the abundance of these species, increased predation by native species seems unlikely. The most likely candidate for increased predation by a non-native predator is trout. Both Rainbow (*Oncorhynchus mykiss*) and Brown (*Salmo trutta*) trout have been introduced at high rates into many streams containing both declining (Alsup 2005) and relatively healthy (USFWS 2001) populations. Although trout have been stocked in Missouri since the late 1800s, the numbers of trout in Missouri streams has increased by approximately three-fold since the 1960s (Alsup 2005). Each year, about one million trout are stocked in several trout parks and management areas in Missouri streams, and over half of these sites are on streams historically occupied by Hellbenders (Alsup 2005). If trout are predators of Hellbenders, this high rate of introduction could potentially have a negative impact on Hellbender population sizes.

With respect to trout, one difference between eastern and western populations of Hellbenders is that many of the eastern streams also are home to a native salmonid, the Brook Trout (*Salvelinus fontinalis*), whereas streams in Missouri have no native trout. In 2002, we were fortunate to collect clutches of eggs from a population in North Carolina (which co-occurs with the native Brook Trout) and from populations of Ozark and Eastern Hellbenders in Missouri for use in another study (Unger 2003; one clutch per population). After rearing larvae in captivity for approximately six months, we tested to see



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Hellbender embryos are nourished by the yolk inside their eggs.



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A six-month-old larva uses its large external gills for respiration.

whether they would perform fright behavior (reduced activity) to the scent of Brown Trout. The populations differed in their responses to the trout stimuli, with larvae from the North Carolina population showing stronger fright responses than larvae from the declining Missouri populations (average fright scores: NC = 210, MO-Ozark = -50, MO-eastern = 90; ANOVA, $P < 0.05$). The populations did not differ with respect to responses to a control stimulus from non-predatory fish (Stonerollers, *Campostoma anomalum*; NC = 10, MO-Ozark = 90, MO-eastern = -20; $P > 0.05$). These results suggest that introduced trout may present more of a problem to larval Hellbenders from declining populations in Missouri (where there are no native trout) than to the more stable population in North Carolina. Note, however, that these data should be interpreted with caution because only one clutch was tested from each population.

Do larvae from declining populations show reduced responses to predators in general or is the reduced response specific to introduced predators? Recent laboratory studies by Gall (2008) extended the above study by examining fright responses to native and non-native fishes by larvae from multiple clutches of eggs gathered from the declining Missouri populations. The data revealed that Hellbender larvae may be especially vulnerable to trout; as in the preliminary study, larvae exhibited relatively low levels of fright (reduced activity) when exposed to trout chemical cues. In contrast, larvae reacted with stronger antipredator behavior when exposed to cues from native fishes, indicating that they are capable of recognizing and responding to native predatory fishes.



Captive-reared Hellbenders in an aluminum raceway.

Captive Breeding and Rearing

Because of the Hellbender's declining populations, the establishment of captive breeding and head-starting programs has been suggested (Mayasich et al. 2003). The Ron Goellner Center for Hellbender Conservation at the St. Louis Zoo has undertaken intensive captive breeding efforts (www.stlzoo.org/wildcareinstitute/hellbendersinmissouri/); adult Hellbenders recently have deposited eggs in an artificial raceway, but so far none have been successfully fertilized either by captive males or through attempted artificial insemination (M. Wanner, pers. comm.). In addition to the captive breeding attempts, The Goellner Center also reared juveniles resulting from clutches collected from the wild by Unger (2003); these "head-started" juveniles were released and are currently being monitored by radio-telemetry (Briggler 2007). However, one concern upon release of captive-reared Hellbenders is that even large juveniles could still be quite vulnerable to predation by large trout. Some head-starting programs for other aquatic species have done a pre-release predator training to help increase survival in the wild (e.g., salmon: Berejikian et al. 1999; trout: Mirza and Chivers 2000). Because of Gall's (2008) findings that larvae did not exhibit high levels of fright to trout, we recently attempted to train larvae to fear trout. Using a methodology that could be implemented in future head-starting programs for Hellbenders, we demonstrated that larvae can learn to associate higher levels of danger with chemical cues from trout (Crane and Mathis, unpubl. data).

Declining Reproduction?

Low recruitment in declining populations also could be explained by decreased reproduction. Unger (2003) investigated reproduction by males in declining and healthy populations. In the first year of the study, only two males in his samples from the declining (Missouri) populations produced any milt, and both semen samples were clumpy with no apparent sperm motility. In the second year, samples were more normal in appearance, but males from declining populations had significantly lower sperm counts than Hellbenders from more stable populations in Georgia and North Carolina (Unger 2003). One hypothesis for the cause of these reproductive problems is poor water quality. Because Hellbenders are fully aquatic and respire almost entirely through their skin (Guimond and Hutchison 1973), they easily can be affected by stream inputs from pesticides, herbicides, agriculture, and erosion. Additionally, Hellbenders are long-lived (30+ years) and pollutants likely accumulate and remain in their tissues for many years (Stebbins and Cohen 1995). In Unger's (2003) study, Hellbenders from Georgia and North Carolina occupied streams in national forests, whereas the Hellbender sites in Missouri are surrounded predominantly by land used for agriculture. Although the nutrient concentrations in Missouri streams likely have not changed much in the past 20–30 years, Hellbenders in Missouri streams are exposed to a variety of organic chemicals that could negatively affect reproduction (Solis et al. 2007). These chemicals (industrial chemicals, plant sterols, and herbicides) at higher levels have been found to have estrogenic effects in shorter-lived amphibians (Mackenzie et al. 2003, Lee et al. 2005). When these chemicals became prevalent in Missouri streams and what species-specific effects they have on Hellbenders is unknown (Solis et al. 2007).

The outlook for the Ozark Hellbender and some populations of the Eastern Hellbender continues to look bleak, but the good news is that progress in conservation efforts is being made. These efforts are being spearheaded by individuals who contribute a wide range of expertise to the problem, including academic researchers, state and federal government biologists, zoo biologists, and conservation education specialists. Captive-rearing and breeding efforts may be our best chance to save the declining Hellbender populations, and efforts by researchers to better understand the plethora of causes responsible for the decline will hopefully enable us to protect the remaining healthy populations from the same threats.



The activity of Hellbenders exposed to different treatments is monitored in the lab.



A wild-caught Hellbender measured in the field.



Hellbender sperm collected from an adult in a healthy population.

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Literature Cited

- Alsop, K.D. 2005. An investigation of the potential threats of nonnative trout on Eastern (*Cryptobranchus alleganiensis alleganiensis*) and Ozark (*Cryptobranchus alleganiensis bishopi*) Hellbender decline. Unpublished M.S. thesis, Saint Louis University, Missouri.
- Berejikian, B.A., R.J.F. Smith, E.P. Tezak, S.L. Schroder, and C.M. Knudsen. 1999. Chemical alarm signals and complex hatchery rearing habitats affect anti-predator behavior and survival of Chinook Salmon (*Oncorhynchus tshawytscha*) juveniles. *Canadian Journal of Fisheries and Aquatic Sciences* 56:830–838.
- Berger, L., R. Speare, P. Daszak, D.E. Green, A.A. Cunningham, C.L. Goggin, R. Slocombe, M.A. Ragan, A.D. Hyatt, K.R. McDonald, H.B. Hines, K.R. Lips, G. Marantelli, and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Science, USA* 95:9031–9036.

- Briggler, J. 2007. MDC Resource Science. Science Notes. Hellbender Recovery Actions in Missouri. Missouri Department of Conservation (MDC). Volume 2(15).
- Briggler, J., K.A. Larson, and K.J. Irwin. 2008. Presence of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) on Hellbenders (*Cryptobranchus alleganiensis*) in the Ozark highlands. *Herpetological Review* 39:443–444.
- Gall, B. 2008. Predator-prey interactions between Hellbenders (*Cryptobranchus alleganiensis alleganiensis* and *C. a. bishopi*) and native and nonnative fishes. Unpublished M.S. Thesis, Missouri State University, Springfield, Missouri.
- Guimond, R.W. and V.H. Hutchison. 1973. Aquatic respiration: An unusual strategy in the Hellbender *Cryptobranchus alleganiensis alleganiensis* (Daudin). *Science* 182:1263–1265.
- Hiler, W.R., B.A. Wheeler, and S.E. Trauth. 2005. Abnormalities in the Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*) in Arkansas: A comparison between two rivers with a historical perspective. *Journal of the Arkansas Academy of Science* 59:88–94.
- 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.
- IUCN/SSC Conservation Breeding Specialist Group. 2006. Hellbender population and habitat viability assessment. Apple Valley, Minnesota.
- Lee, S.K. and N.D.R. Veeramachaneni. 2005. Subchronic exposure to low concentrations of di-n-butyl phthalate disrupts spermatogenesis in *Xenopus laevis* frogs. *Toxicological Science* 84:394–407.
- Mackenzie, C.A., M. Berrill, C. Metcalfe, and B.D. Pauli. 2003. Gonadal differentiation in frogs exposed to estrogenic and antiestrogenic compounds. *Environmental Toxicology and Chemistry* 22:2466–2475.
- Mayasich, J., D. Grandmaison, and C. Phillips. 2003. Eastern Hellbender status assessment report. Final report, U.S. Fish and Wildlife Service, Region 3. Fort Snelling, Minnesota.
- Miller, B.T., and J.L. Miller. 2005. Prevalence of physical abnormalities in Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) populations of middle Tennessee. *Southeastern Naturalist* 4:513–520.
- Mirza, R.S. and D.P. Chivers. 2000. Predator-recognition training enhances survival of Brook Trout: Evidence from laboratory and field enclosures. *Canadian Journal of Zoology* 78:2198–2208.
- Nickerson, M.A., K.L. Krysko, and R.D. Owen. 2002. Ecological status of the Hellbender (*Cryptobranchus alleganiensis*) and the Mudpuppy (*Necturus maculosus*) salamanders in the Great Smoky Mountains National Park. *Journal of the North Carolina Academy of Science* 118:27–34.
- Nickerson, M.A. and C.E. Mays. 1973. The Hellbenders: North American Giant Salamanders. *Milwaukee Public Museum Publications in Biology and Geology* 1:1–106.
- Peterson, C.L. 1985. Comparative demography of four populations of the Hellbender, *Cryptobranchus alleganiensis*, in the Ozarks. Unpublished Ph.D. Dissertation, University of Missouri, Columbia.
- Petranka, J.W. 1998. *Salamanders of the United States and Canada*. Smithsonian Institution Press, Washington, D.C.
- Sabatino, S.J. and E.J. Routman. 2008. Phylogeography and conservation genetics of the Hellbender salamander (*Cryptobranchus alleganiensis*). *Conservation Genetics* 10.1007/s10592-008-9655-5.
- Skerratt, L., L. Berger, R. Speare, S. Cashins, K. McDonald, A. Phillott, H. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134.
- Solis, M.E., C.C. Liu, P. Nam, D.K. Niyogi, J.M. Bandeff, and Y.W. Huang. 2007. Occurrence of organic chemicals in two rivers inhabited by Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*). *Archives of Environmental Contamination and Toxicology* 53:426–434.
- Stebbins, R.C. and N.W. Cohen. 1995. *A Natural History of Amphibians*. Princeton University Press, Princeton, New Jersey.
- Unger, S. 2003. Sperm production and larval development in Hellbenders (*Cryptobranchus alleganiensis alleganiensis* and *C. A. Bishopi*): A comparison of declining and stable populations. Unpublished M.S. Thesis, Southwest Missouri State University, Springfield, Missouri.
- U.S. Fish & Wildlife Service, Southeast Region. 2001. Economic effects of trout production by national fish hatcheries in the Southeast. U.S. Fish and Wildlife Service, Atlanta, Georgia.
- 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.