

AHSLC Seed Grant Report 2006

Ecology, conservation and genetic diversity of hellbenders in the Great Smoky Mountains National Park

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The purpose of this study was to continue an ongoing study surveying the river systems in the Great Smoky Mountains National Park (GSMNP) and southern Cherokee National Forest to identify the distribution of eastern hellbenders, and then conduct population studies to determine the long-term viability of these populations. This is an important first step in gaining sufficient data to determine an appropriate level of state protection for hellbenders in Tennessee.

The hellbender *C. alleganiensis* is one of the largest salamander found in North America and has suffered severe declines across much of their range (Bury et al. 1980, Williams et al. 1981). Of the 17 states with hellbender populations, most classify the species as endangered, rare, or a species of concern, and are implementing tracking programs to monitor populations. These salamanders are found only in large, fast-flowing, highly oxygenated streams, and are extremely sensitive to siltation, dam construction, and acid mine drainage (Petranka 1998). In Tennessee, hellbenders are currently classified “in need of management”, and in North Carolina as “species of special concern”. In both cases, there is concern over the status of populations, however insufficient data has been collected to determine whether state protection is required. It is believed that hellbenders may be locally abundant in some river systems, but absent in most. Unfortunately current presence/absence data is particularly scarce in southern Appalachia, so the true distribution of hellbenders in the region’s river systems is not known, and there is even less information on the long-term viability of any populations (Herrig, pers. comm.).

Much of the best potential aquatic habitat for hellbenders in the region is found in eastern Tennessee and western North Carolina, in the Great Smoky Mountains National Park GSMNP (Nickerson et al. 2002), and the north and south sections of the Cherokee National Forest. These

areas are also protected from much of the human impact implicated in declines elsewhere (Wheeler et al. 2003).

Survey Sites and Methods

During 2006, we continued surveys begun in 2004. In the GSMNP we surveyed the Little River (210 person hours), East Prong of the Little Pigeon River (4 person hours), Hazel Creek (20 person hours), and Oconaluftee River (12 person hours). In the Cherokee NF we surveyed the Hiwassee River (95 person hours) and the Tellico River (12 person hours).. The methods involved searching several sections of each river, carefully turning rocks on the stream bed to reveal hellbenders sheltering beneath. We recorded location (using GPS), mass, length and sex (when possible) of every hellbender we found, and then injected a PIT (passive integrated transponder) tag in all adults and sub-adults for permanent identification (gilled juveniles are too small to PIT tag). We used capture rate (hellbenders caught per person search hour) to produce a relative estimate of density. Tables 1 (Great Smoky Mountains NP) and 2 (Cherokee NF) summarize all of the capture data to date since 2004.

Distribution and Abundance

Table 1 –Great Smoky Mountains NP hellbender capture data

	Hellbenders captured	Search Effort (person hours)	Mean Capture Rate (per person hour)
Little River	240	565	.43
Deep Creek	7	47	0.15
Abrams Creek	1	31	0.03
Oconaluftee River	4	19	.21
E. Prong Little Pigeon	0	19	0
W. Prong Little Pigeon	0	15	0
Hazel Creek	0	20	0

Table 2 – Cherokee NF hellbender capture data

	Hellbenders captured	Search Effort (person hours)	Mean Capture Rate (per person hour)
Hiwassee River	263	310	.85
Tellico River	15	29	.52
Citico River	0	35	0

Table 3 – Mark/Recaptures

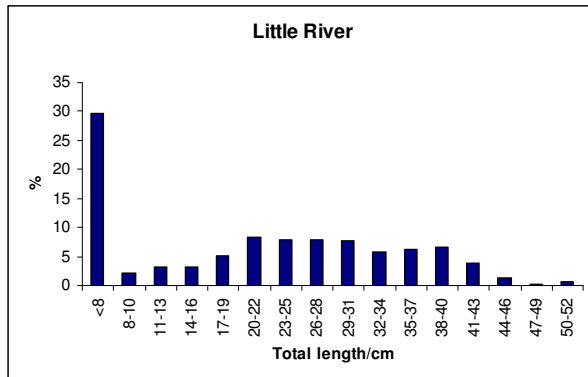
	Hellbenders tagged	Hellbenders recaptured
Little River	134	10
Hiwassee River	135	29
Tellico River	13	2

Population Structure

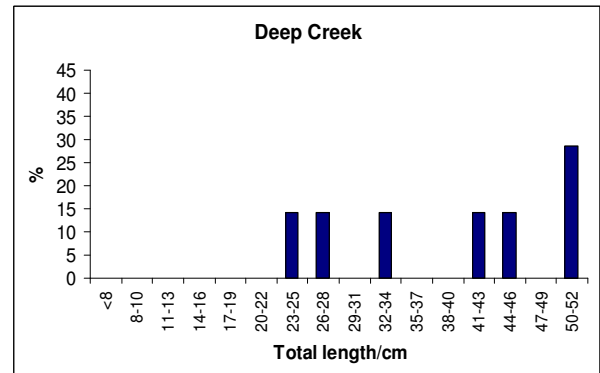
In addition to mapping the distribution of hellbender populations, we wish to assess the health/viability of populations. An important measure is the population structure. If all age classes are present, we can conclude that there has been successful reproduction for many years, and if gilled larvae (< 1 year old) are present, successful reproduction is still occurring. We do not yet have a direct measure of age, therefore we use total body length to construct frequency histograms of size classes (Fig. 1)

Figure 1: Frequency histogram of hellbender total length, Little River (a) Deep Creek (b), Oconaluftee River (c) Hiwassee River (d), and Tellico River (e).

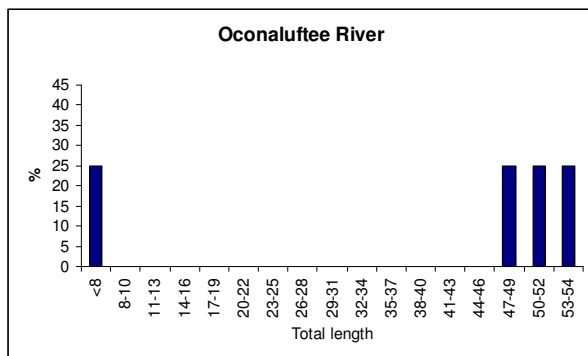
1(a)



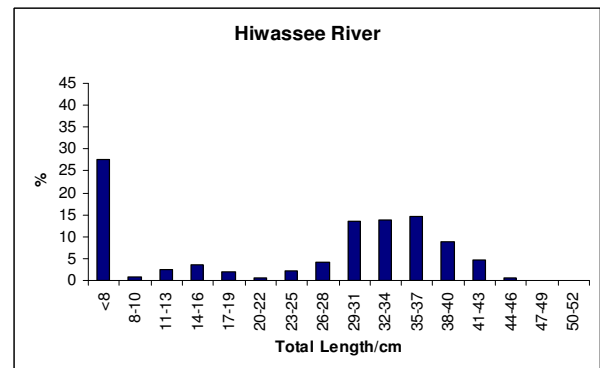
1(b)



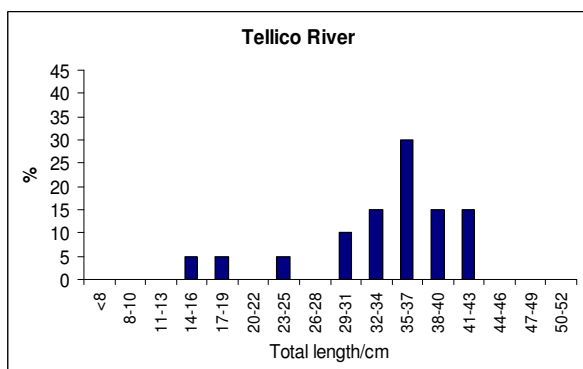
1(c)



1(d)



1(e)



Conclusion

Our surveys to date have identified large, viable populations in the Little and Hiwassee Rivers. All size classes are represented, including the larval stage. Of the two, the Hiwassee appears to have the highest population density. We have recaptured 29 hellbenders in the Hiwassee and 10 in the Little River over the last two summers, unfortunately they are all in a rather restricted size range (30-42cm total length). Therefore the recapture data are not yet very informative on growth rates, survival etc. More than 50% of the hellbenders we have caught are in this size range, and gilled larvae (which make up 25%) are not tagged, so it is not surprising that these mid-sized hellbenders are the most likely to be recaptured.

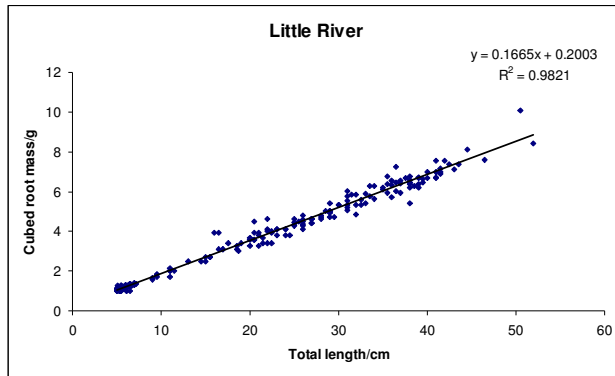
We have spent much less time surveying, Deep Creek, Oconaluftee and Tellico however our preliminary data suggest that Tellico may possess a viable population since a diverse range of size classes with the exception of gilled larvae have been found. The population density (based on capture rate) would also appear to be similar to the Little River. Oconaluftee River appears to have a very low population density, however the capture of one gilled larva suggests that there is current reproduction. Deep Creek appears to be dominated by older individuals – as yet we have not found larvae or juveniles. Only one large hellbender has been found in Abrams Creek. To date, we have not yet found any hellbenders in Little Pigeon River, Hazel Creek and Citico River. This is very concerning since there are previous records of hellbenders in these rivers.

Body Condition

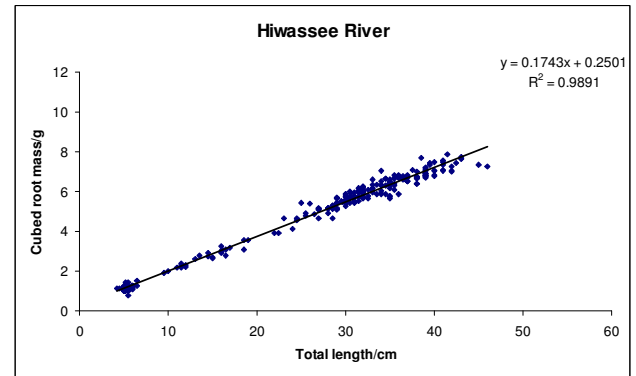
Hellbender populations in decline are likely to show a decrease in body condition. We constructed a measure of body condition by plotting cube root of body mass against total body length. This produces a highly significant straight line regression (Fig. 2)

Figure 2 – Body Condition of hellbenders from the Little River (a) and Hiwassee River (b)

2(a)



2(b)



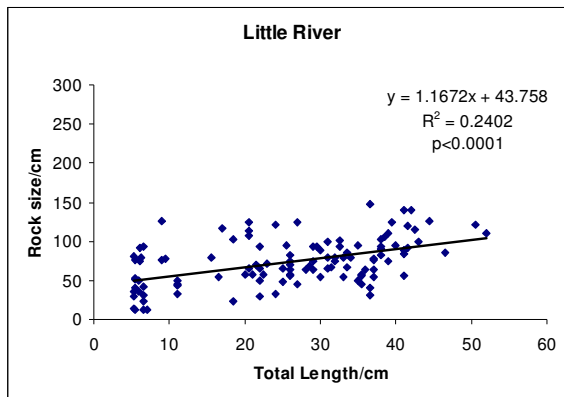
There are no previous data on body condition to compare with our data to test for any decline in condition, so our data provide a baseline for future studies. Interestingly, both the slope and intercepts of the lines for the two rivers are significantly different (slope: $t_{(1),471} = 4.13$, $p < 0.0005$, Intercept: $t_{(1),472} = 9.065$, $p < 0.0005$). This suggests that hellbenders in the Hiwassee are slightly heavier for any given length, and increase mass at a faster rate (with respect to body length). This may be attributable to higher crayfish densities in the Hiwassee (2.3 crayfish/m^2) than in the Little River ($0.47 \text{ crayfish/m}^2$). These crayfish densities were estimated using 1 m^2 quadrats along 25m transects of streambed

Rock/Habitat Use

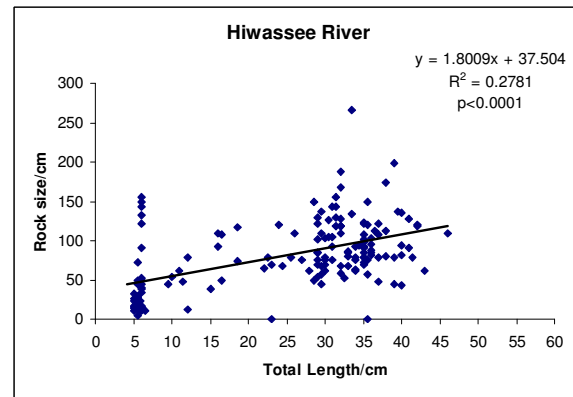
Most of the hellbender literature states that there is no correlation between hellbender body length and the size of the rock it was found under (e.g. Humphries and Pauley 2005). However these studies typically do not include larval hellbenders. Since we have been able to capture a large number of larvae from the Hiwassee and Little Rivers, we were interested to see how our data might compare with the published studies. We measured the largest width of each rock under which a hellbender was captured, and plotted rock size against total body length (Fig 3).

Figure 3 – Rock use by hellbenders in the Little River (a) and Hiwassee River (b)

3(a)



3(b)



In both rivers, there is a strongly significant relationship between body size and rock size. Small hellbenders, and especially larvae, are generally found under much smaller rocks than large hellbenders. In the Hiwassee, many larvae were found in shallow glides consisting primarily of a cobblestone bottom. The slope of the lines of the two rivers are not significantly different, however the intercepts are (slope: $t_{(2),257} = 1.648$, ns, intercept: $t_{(2),257} = 2.374$, $p < 0.05$). This suggests that hellbenders in the Little River tend to use larger rocks than similar sized hellbenders in the Hiwassee. The Little River is susceptible to powerful flooding, and it may be important to choose rocks that remain in position. The shape of the ropes is also somewhat different since most rocks in the GSMNP are metamorphic sandstones which are quite rounded, while rocks in the Hiwassee tend to be non-metamorphic sandstone slabs that have fractured along cleavage planes.

Tissue Analysis – Pesticides and Mercury

Concerns have also been raised that sub-lethal effects of pesticide residues in the environment may be contributing to general amphibian declines (Corn 2000), including hellbenders. Black-bellied salamanders (*Desmognathus quadramaculatus*) collected from several drainages in the GSMNP, contained residues of several different pesticides including DDT, chlordane, chlorpyrifos, endosulfan and atrazine (Freake and Lindquist, in prep). Chlorpyrifos, endosulfan and atrazine are still in current use and are moderately-highly toxic to amphibians; even exposure

to extremely low levels has been shown to cause behavioural and developmental harm. (Mazanti 1999; Hayes et al 2002). The GSMNP also experiences unusually high mercury deposition from coal power stations, and concerns have been raised about mercury accumulations in fish and amphibians within the park (Jim Renfro, air quality specialist, GSMNP Air Quality Program, pers. comm.). We collected tail tissue from 8 hellbenders in the Hiwassee and 4 individuals in the Little River, which were frozen and sent to the Virginia Tech Pesticide Residues lab and School of Veterinary Medicine. The tissue from each river was combined to produce two samples from the Hiwassee and 1 from the Little River, which were subjected to general screen pesticide and mercury analysis (Table 4)

Table 4 – Mercury and Pesticide Residues in hellbender tail tissue

	p,p-DDE/ppb	Mercury/ppb*
Hiwassee 1	2.54	33
Hiwassee 2	2.85	ND
Little River	4.35	39

Our data indicate very low levels of DDT metabolite and mercury. These levels are similar to background levels found in most vertebrates, and are unlikely to represent a significant biological harm (Steve Holladay, Virginia Tech School of Veterinary Science, personal communication).

Tissue Analysis - Histology

Toes were also collected from hellbenders captured in the Hiwassee River for skeletochronology (Halliday and Verrell 1988, Kumbar and Pancharatna 2002). A number of toe samples from previous years were fixed, decalcified and embedded in wax for sectioning at a local pathology lab. We have begun sectioning and staining them with Hematoxylin and Eosin. Unfortunately the samples to date did not show clear lines of arrested growth (LAG) to permit reliable aging. We are currently trying different decalcification protocols to ensure that LAG details are not lost.

Tissue Analysis - DNA

Previous studies indicate that genetic diversity in hellbender populations is low, however populations from different rivers tend to have unique haplotypes, suggesting little if any gene flow between rivers (Routman 1993, Routman et al. 1994). Only in the Tennessee Valley was there some evidence for gene flow. Since the impoundment of many rivers in the watershed by TVA, historical migration between populations may be currently reduced, and contributing to a decrease in overall population stability. Routman's studies were conducted at a coarse scale over the entire range of hellbenders, with only a few rivers from each geographic areas sampled. We are in the process of collecting samples from as many local populations as possible in east Tennessee, west N. Carolina and northern Georgia. We are using mitochondrial sequences to assess genetic diversity and gene flow between rivers. Using primers developed by Routman, we have successfully amplified cytochrome b (~1000 bp) and cytochrome oxidase I (~900 bp) fragments from hellbender tissue from Tennessee (Little, Hiwassee, Tellico and Tellico Rivers), N. Carolina (Oconaluftee, Deep Creek, Looking Glass Creek) and Georgia (Hiwassee River, Milky Creek). We are also attempting to amplify two other mitochondrial fragments (d loop and dehydrogenase subunit 4). We will shortly begin mapping the fragments using restriction site analysis to identify haplotypes found within and between rivers.

Conclusions

Given the historical decline in hellbenders over their entire range, our research is providing extremely valuable information on populations in the southern Appalachians that have never received intensive study in the past. Our data are providing the most accurate information on local distribution available, and are providing critical insights into the viability of these populations. There is considerable interest in hellbenders amongst the herpetology and conservation communities. I have presented our data at the Joint Meeting of Ichthyologists and Herpetologists, New Orleans LA, July 2006, and will also be presenting at the 3rd Hellbender Symposium in Wheeling WV, June 2007. The project has also provided valuable research opportunities for Lee University undergraduates, and educational opportunities for school students through our close relationship with the Great Smoky Mountains Institute at Tremont, and field excursions with local schools such as Tellico Plains High School.

As the surveys continue over the next few years, the population structures at some of our lesser studied sites will become clearer, and mark-recapture data will provide much more accurate estimates of population density, as well as providing insights into mortality, immigration and emigration rates. At the same time our molecular genetics study should allow us to estimate levels of genetic diversity in our populations, and identify whether populations have been linked historically through migration. Meanwhile, we are sharing our data as we collect it with the National Park Service, National Forest Service, and Tennessee Wildlife Resources Agency, and it is already helping them in planning appropriate management strategies for hellbenders in this region.

References

- Bury , R. B., Dodd, C. K., Jr. and G. M. Fellers. 1980. Conservation of the amphibia of the United States: a review. U. S. Department of the Interior, Fish and Wildlife Service, Washington, D. C., Resource Publication 134: 1-34.
- Corn, P. S. 2000. Amphibian declines: review of some current hypotheses. In *Ecotoxicology of Amphibians and Reptiles* (Sparling D.W., Lindner G. & Bishop C. A. Eds). SETAC.
- Halliday, T. R. and P. A. Verrell. 1988. Body size and age in amphibians and reptiles. *J. Herpetol.* 22: 253-256.
- Hayes T.B., Collins A., Lee M., Mendoza M., Noriega N., A. Stuart A., and Vonk A. (2002) Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. *Proc. Nat. Acad. Sci.* 99: 5476-5480
- Humphries W. J. and Pauley T. K. (2005) Life History of the Hellbender, *Cryptobranchus alleganiensis*, in a West Virginia Stream. *American Midland Naturalist* 154:135–142
- Kumbar, S. M. and K. Pancharatna. 2002. Annual growth layers in the phalanges of the Indian skipper frog *Rana cyanophlyctis* (Schn.). *Copeia* 2002: 870-872.
- Mazanti L. 1999. The effects of atrazine, metalochlor and chlorpyrifos on the growth and survival of larval frogs under laboratory and field conditions. Ph.D. Thesis. University of Maryland.

- 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. J. N. Car. Acad. Sci. 118:27-34.
- Petranka, J.W. 1998. *Salamanders of United States and Canada*. Smithsonian Institution Press, Washington, D.C.
- Routman, E.J. 1993. Mitochondrial DNA variation in *Cryptobranchus alleganiensis*, a salamander with extremely low allozyme diversity. Copeia. 1993:375-384.
- Routman, E.J., R. Wu, and A.R. Templeton. 1994. Parsimony, molecular evolution, and biogeography: the case of the North American giant salamander. Evolution. 48:1799-1809.
- Wheeler B. A., Prosen E., Mathis A, and Wilkinson R. F. (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. Wildl. Soc. Bull. 9(2): 94-100.

Appendix

Figure 4 – Great Smoky Mountains NP survey sites

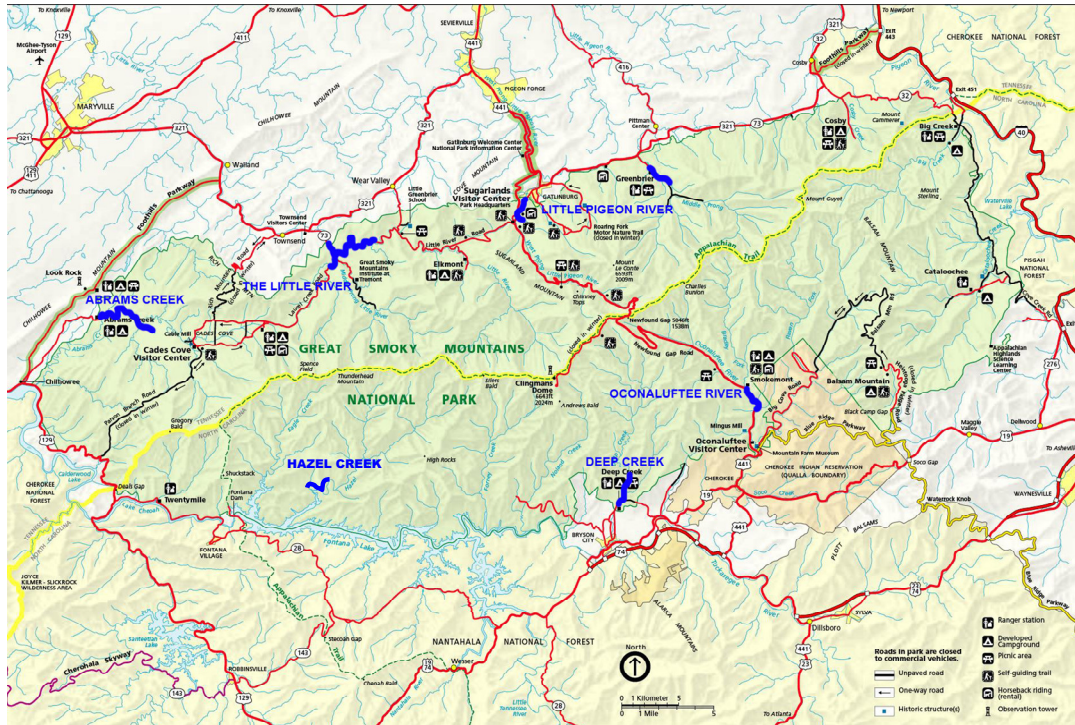


Figure 5 – Cherokee NF Survey sites

