Herpetological Conservation and Biology 13(3):598–608.
Submitted: 1 February 2018; Accepted 5 September 2018; Published 16 December 2018.

A Protocol for Quantifying Hellbender Abundance and In-Stream Habitat

M. Worth Pugh[1,2,4], *Thomas Franklin*[1,3], *Lynn Siefferman*[1], *and Michael M. Gangloff*[1]

[1]*Department of Biology, Appalachian State University, 572 River Street, Boone, North Carolina 28608-2027, USA*
[2]*Department of Biological Sciences, The University of Alabama, Tuscaloosa, Alabama 35487-0340, USA*
[3]*United States Department of Agriculture, Forest Service, National Genomics Center for Wildlife and Fish Conservation, Rocky Mountain Research Station, Missoula, Montana 59801, USA*
[4]*Corresponding author: mwpugh@ua.edu*

***Abstract*.—Effective habitat and species monitoring programs require robust and repeatable estimates derived from standardized protocols. Hellbenders (*Cryptobranchus alleganiensis*) are large, long-lived salamanders endemic to highland streams in central and eastern North America. Based on historical data, it is apparent that Hellbender populations are undergoing significant, wide-spread declines; however, the ability of researchers to detect declines is limited because there has been little effort to standardize surveys and virtually no quantitative habitat data are collected during surveys. Here, we assess the efficacy of a spatially constrained transect-based method to capture Hellbenders and describe habitat conditions among years. We compared results to conventional snorkel/rock-turning surveys and tested the consistency of habitat parameters using intra-class correlations. Although the differences were not statistically significant, spatially constrained surveys captured 25% more animals and produced relative abundance estimates that were 107% higher than unstandardized surveys. By constraining surveys and carefully recording effort, we ensured technicians would search study reaches more effectively and find Hellbenders in habitats that may have been overlooked by unconstrained surveys. Intra-class correlations demonstrated that some physical habitat conditions remained consistent between years whereas others were much more variable reflecting the year-to-year variability inherent to stream ecosystems. By constraining Hellbender surveys in time and space, researchers can provide more informative estimates of abundance and habitat suitability that will improve the ability of monitoring programs to detect changes in the range and population sizes of these large but cryptic aquatic salamanders.**

Key Words.—conservation; *Cryptobranchus*; in-stream habitat; quantification; search-effort; survey

Introduction

Monitoring trends in wild populations coupled with rigorous habitat assessment are fundamental to conservation biology (Yoccoz et al. 2001). Nonetheless, because protocols are frequently not standardized, many stakeholders (i.e., state and federal agencies, private land-owners, academics) produce data that are not easily comparable and thus may not accurately represent population trends or properly document the influences of habitat on detectability and site occupancy (Rödel and Ernst 2004). This presents a significant obstacle for stakeholders who are responsible for making informed management decisions that are potentially biased by results of unstandardized surveys that may not accurately quantify abundance or occupancy. Moreover, although detection of species declines is often the priority of monitoring programs, the underlying causes of species decline such as habitat degradation are often of greater interest, yet few researchers use quantitative methods to assess local habitat conditions. Therefore, it is important to standardize both survey efforts and evaluation of local habitat particularly for species in decline or of special concern (Brower and Zar 1998).

Hellbenders (*Cryptobranchus alleganiensis*; Fig. 1) are large (maximum total length about 74 cm) aquatic salamanders endemic to mountain and upland streams in eastern and central USA (Nickerson and Mays 1973; Petranka 1998). Hellbenders exhibit cryptic coloration and occupy cavities under large rocks in cool fast-flowing streams (Smith 1907; Hillis and Bellis 1971; Nickerson and Mays 1973; Nickerson and Krysko 2003). In recent decades numerous researchers have reported rapid, widespread declines in Hellbender populations (Mayasich et al. 2003; Briggler et al. 2007; Foster et al. 2009; Burgmeier et al. 2011; Pitt et al. 2017). Plausible causes of these declines include habitat degradation due to pollution and land-use change (Wheeler et al. 2003; Quinn et al. 2013; Pugh et al. 2016; Pitt et al. 2017) over-exploitation (Nickerson and Briggler 2007), needless killing (Reimer et al. 2013), and pathogens including *Ranavirus* and *Batrachochytrium dendrobatidis* (Bodinof et al. 2011; Souza et al. 2012; Williams and Groves 2014).

Figure 1. An adult Hellbender (*Cryptobranchus alleganiensis*) captured at one of the study sites in the Watauga River Drainage in northwestern North Carolina and eastern Tennessee, USA. (Photographed by M. Worth Pugh).

Recent efforts to understand and mediate Hellbender decline have primarily involved disease testing, captive breeding, deploying artificial nest rocks, and population monitoring (Briggler et al. 2007). Although state wildlife agencies conduct regular monitoring of Hellbender populations, to date there is no established standardized protocol that incorporates quantification of Hellbender abundance and physical habitat parameters. This is surprising considering there are several proposed methods for fishes (Dauwalter et al. 2003; Meador et al. 2003; McCluskey and Lewison 2008), mussels (Huang et al. 2011; Hart et al. 2016), and other aquatic salamanders (Crawford and Semlitsch 2007; Greene et al. 2008) that occupy similar, or often, the same habitats. Because an understanding of the influences of local habitat on Hellbender populations is often a significant component of questions related to Hellbender management, a lack of standardized and quantitative habitat data is an impediment to investigation of range-wide trends in Hellbender populations. Adoption of a standardized protocol may improve the ability for range-wide comparison of data and population trends in Hellbender populations particularly in the many cases where neighboring states share parts of the same river drainage.

Herein we describe a spatially constrained sampling protocol to inventory Hellbender populations and a protocol to quantify habitat parameters (Pugh et al. 2013, 2016; Franklin 2016). We describe our methodology and compare number of captures using this standardized method with unstandardized conventional timed-search surveys. We also examine the consistency of our habitat characterization method between field seasons. We predicted that our transect survey would increase Hellbender captures and estimates of Hellbender abundance and that our habitat characterization would produce consistent results among field seasons.

Materials and Methods

Hellbender surveys.—We conducted Hellbender surveys at 20 sites in the Watauga River Drainage in northwestern North Carolina and eastern Tennessee, USA, during 2011 and 2012 using a conventional timed search in 2011 and a transect-based method in 2012. We conducted all surveys from May to August and ceased surveys in September due to the start of the breeding season. We compared number of captures and estimates of relative abundance of Hellbenders at sites where we detected Hellbenders one or both years (2011 and 2012). Sites consisted of a 150-m stream reach divided by cross-channel transects at 10-m intervals (n = 16 per site). We then surveyed study reaches one section at a time and we recorded the total effort expended within each section. Prior experience in these watersheds indicates that a 150-m search area is large enough to contain at least one or two Hellbenders, and in some cases, a 150-m reach may contain as many as 20 individuals (Pugh et al. 2016).

After delineating transects, we conducted either a conventional timed search (2011) or a transect survey (2012). We searched for Hellbenders by turning medium- to large-sized cobbles and boulders by hand or using log peaveys (Nickerson and Krysko 2003). We located Hellbenders by feeling underneath cover rocks with our hands or visually detecting them when water clarity under the rock allowed. Additionally, we would occasionally encounter Hellbenders in bedrock seams or moving across the substrate. We captured Hellbenders by hand or allowed them to swim into dip-nets set immediately downstream of cover rocks. Survey teams consisted of a minimum of three persons (two snorkelers and one rock lifter) and up to 12 technicians at a time. Although in larger streams more searchers would be appropriate, our sites were located in headwater regions and the search area could become unacceptably crowded if we exceeded this number potentially skewing our estimates of abundance. The number we felt was most convenient was six technicians where four conduct the survey and two are available to process animals (i.e., collect body condition data). After processing, we always returned Hellbenders to their site of capture and released them in front of their cover rock marked with flagging tape tied to small metal washers.

During conventional timed searches, we attempted to search the entire 150-m reach using a single, continuous search effort. If we had multiple captures, we would stop time to process animals and resume time once the animals were released. In contrast, during transect surveys, we sampled the study reach by searching each 10-m section between transects (n = 15) as a discrete unit. One technician supervised those searching to make sure that they remained in focal transects and

Figure 2. Field technicians conducting a Hellbender (*Cryptobranchus alleganiensis*) survey using the transect method. Transects are laid every 10 m and the area between two transects were searched independently. (Photographed by Jason Selong).

that all available habitats were surveyed (Fig. 2). After each transect, we would stop and record search time, number of Hellbenders captured, and number of snorkelers before moving on to the next transect. For both survey methods we estimated Hellbender relative abundance as catch per unit effort (CPUE) by dividing the number of Hellbenders encountered at a site by the number of snorkeling technician search hours. Although Hellbenders occasionally evade capture, experienced and well-trained field teams rarely fail to capture detected Hellbenders (Franklin 2016).

Habitat characterization.—We conducted habitat characterization protocols at 20 sites in the Watauga River drainage in 2011 and 2012 and at 10 sites in the New River Drainage in 2014 and 2015 and compared habitat assessments within each drainage between sampling years. We sampled all sites during May-August and we did not sample during periods of high flow to avoid biasing flow, depth, and width measurements of streams. At each transect we measured the wetted channel width of the stream using a meter tape. Along each transect we measured stream depth and current velocity at five equidistant points. We sampled stream substrate by walking along each transect and randomly selecting 25 substrate particles from the front of our shoes every one to two steps or more depending on the width of the stream (Wolman 1954). We measured the maximum diameters of all lithic particles > 2 mm and classified particles > 2 m as boulders. We also classified fine substrates and organic particles as bedrock, silt, sand, organic matter (i.e., leaf pack and aquatic macrophytes) or woody debris. We calculated percentages of non-measurable substrates as the portion of samples in a non-measurable substrate category divided by the total number of samples taken (n = 400 per site). We used habitat data to compute mean wetted width, current velocity, depth, percentage non-measurable substrate parameters as well as mean and median particle size. We added percentage of silt and sand together to obtain an estimate of the proportion of fine substrates present in a site.

Statistical analysis.—We compared the total number of captures and CPUE of Hellbenders from occupied sites in the Watauga River Drainage sampled during 2011 (conventional timed-search surveys) and 2012 (transect surveys) using a paired Wilcoxon Signed Rank Test ($\alpha = 0.05$). We used intra-class correlations (ICCs) to examine consistency of habitat parameters between sampling years in the Watauga River Drainage (2011–2012) and New River Drainage (2014–2015). We separated habitat parameters by year and ICCs were run independently for each parameter and compared using ANOVA ($\alpha = 0.05$). Most habitat data were not normally distributed (Shapiro-Wilkes $P < 0.05$), so we transformed data using a Log_{10} (n + 1) transformation prior to analyses to normalize data (Shapiro-Wilkes $P > 0.05$). Because many of our study streams lacked woody debris and boulders, we removed percentage woody debris from the Watauga Drainage sites and percentage boulder from New Drainage sites prior to analysis because including all these missing data points in our analyses produced highly skewed relationships that were unreliable. In both instances excluded substrate types comprised < 5% of all substrate measurements made across all sites and years. We conducted all analyses in SPSS 24.0 (SPSS Inc., Chicago, Illinois, USA).

Results

We detected Hellbenders at six sites in 2011 and 2012 in the Watauga River drainage. At 66% of sites, we found more Hellbenders using the transect survey method than we had the previous year using the conventional timed-search method. Hellbender capture rates increased by 25% across all sites using the transect method (Fig. 3). This increase included a new detection at one site; however, most of the additional captures occurred at one densely populated site that produced 13 captures in 2011 (conventional timed-search method) and 20 captures in 2012 (transect method). From 2011 to 2012, our estimates of Hellbender abundance (CPUE) increased at 83% of sites using the transect method including one site that produced greater CPUE despite the fact we found fewer Hellbenders in our 2012 survey (Site 5). Additionally, there was a 107% increase in mean CPUE across sites between conventional timed surveys and the transect method (Fig. 4), but differences were not significant (n = 6; $Z = -1.363$; $P = 0.172$).

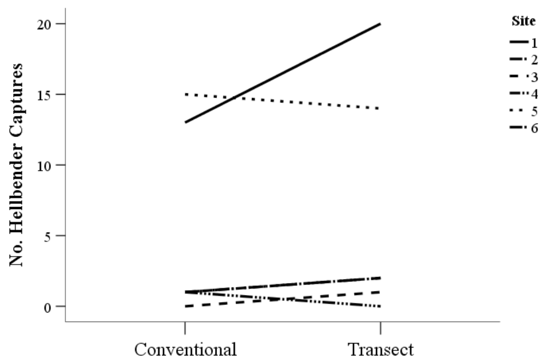

Figure 3. Comparison of Hellbender (*Cryptobranchus alleganiensis*) captures from the Watauga River using the conventional timed survey (2011) and transect methods (2012). Total number of Hellbender captures increased 25% using the transect method versus the conventional timed survey. At Sites 2 and 4 we found one Hellbender using the conventional timed-search method and two using the transect method making the trend lines overlap completely.

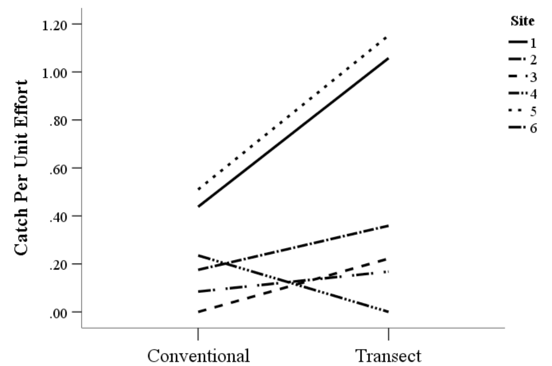

Figure 4. Comparison of Hellbender (*Cryptobranchus alleganiensis*) catch per unit effort (CPUE) using the conventional timed survey (2011) versus the transect method (2012). We calculated CPUE as the number of Hellbender captures divided by the number of person search hours.

Intra-class correlations demonstrated high consistency in stream depth and width measurements in the Watauga Drainage; however, most other habitat parameters were not significantly associated between years (Table 1; Fig. 5). Two habitat parameters, mean and median substrate size, were somewhat repeatable (intra-class coefficients > 0.50) between surveys, although this trend was also not statistically significant (Table 1). In the New Drainage, intra-class correlations revealed that measurements of stream depth, velocity, width, percentage bedrock, percentage organic substrates, and percentage fine substrates were highly consistent between surveys (Table 1; Fig. 6).

Discussion

The survey and habitat assessment methods outlined here have the potential to be useful to researchers interested in obtaining standardized, repeatable, and statistically informative Hellbender abundance estimates as well as quantitative habitat datasets that appear sensitive to annual (and likely long-term) habitat variability. There are numerous reasons why researchers and agencies working to conserve Hellbenders should begin to employ some level of standardization during surveys as many researchers and agencies have for other sensitive aquatic fauna (Dauwalter et al. 2003; Meador et al. 2003; Crawford and Semlitsch 2007; Huang et al. 2011; Hart et al. 2016). First, because standardized surveys may detect more Hellbenders on average compared to unconstrained surveys, they may simply yield more reliable and repeatable population estimates that are comparable across populations making such methods ideal for interpreting range-wide population trends. Focused surveys that yield precise estimates of population size will be needed to track future trends in Hellbender abundance and can be useful even when animals are not detected as they provide some context for the amount of habitat searched. Additionally, because Hellbenders exhibit site fidelity, it should also be possible to adapt this methodology to simplify mark and recapture surveys. Finally, the protocol that we developed incorporates a method for quantifying habitat conditions that is sensitive to site to site and year to year variability. Without reliable and repeatable

Table 1. Intra-class correlations for habitat parameters collected in the Watauga Drainage (2011–2012) and New Drainage (2014–2015). Higher intra-class correlations (ICCs) represent greater consistency between field seasons. *F* and *P* values represent result from comparison using ANOVA. Parameters marked with an asterisk (*) represent a statistically significant ICC.

Drainage	Parameter	ICC	*F*	*P*
Watauga	Log Depth	0.79*	4.72	0.001
	Log Velocity	-0.55	0.65	0.824
	Log Mean Substrate Size	0.51	2.05	0.063
	Log Median Substrate Size	0.50	2.01	0.068
	Log Mean Width	0.97*	36.1	< 0.001
	Log % Bedrock	-0.19	0.84	0.643
	Log % Organic Substrates	0.35	0.74	0.714
	Log % Boulder	-0.11	0.90	0.570
	Log % Fine Substrates	-0.40	0.72	0.722
New	Log Depth	0.92*	12.9	< 0.001
	Log Velocity	-0.32	0.76	0.657
	Log Mean Substrate Size	-0.07	0.94	0.539
	Log Median Substrate Size	0.08	1.09	0.449
	Log Mean Width	0.97*	32.7	< 0.001
	Log % Wood	0.42	1.72	0.217
	Log % Bedrock	0.94*	15.8	< 0.001
	Log % Organic Substrates	0.88*	8.43	0.003
	Log % Fine Substrates	0.89*	8.72	0.002

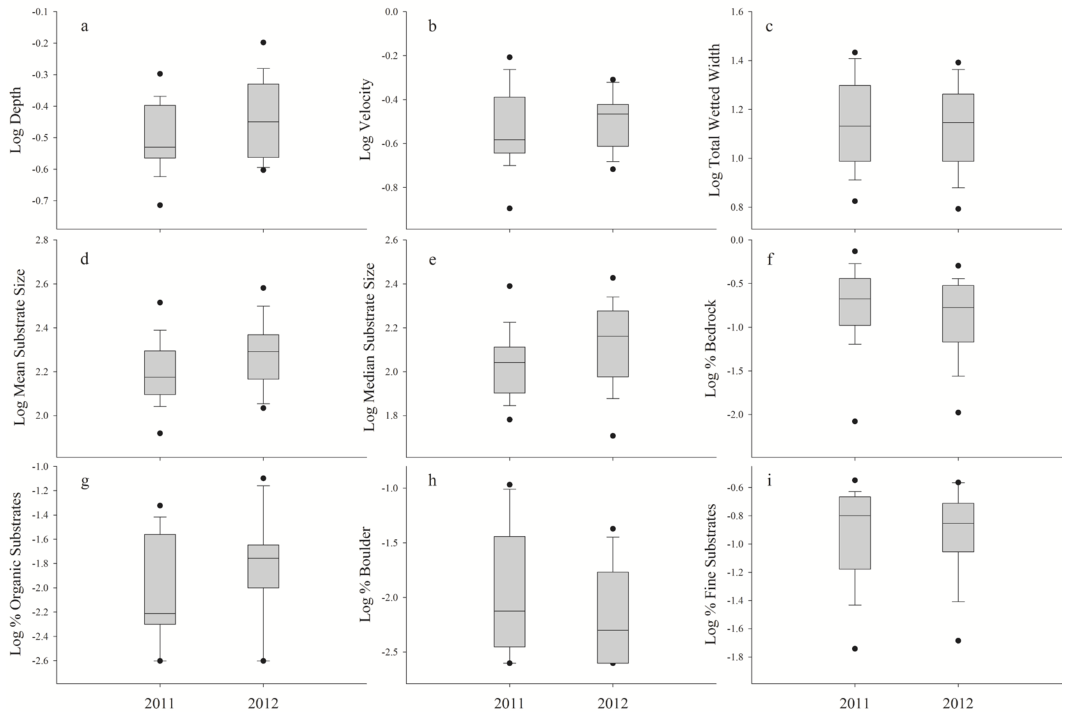

Figure 5. Comparison of physical habitat parameters in the Watauga River Drainage between 2011 and 2012 field seasons. Boxes represent 25th and 75th percentiles and error bars represent 90th percentiles.

estimates of Hellbender detectability combined with physical habitat conditions, the links between changes in habitat conditions and Hellbender populations will likely remain largely speculative (Maddock 1999; Briggler et al. 2007; Pugh et al. 2016; Pitt et al. 2017). Although many researchers collect qualitative habitat data (i.e., visually estimating substrate composition or describing local land-use conditions), these data are essentially unrepeatable and have limited power in statistical analyses. By spending a small amount of extra time (e.g., about 1-2 h per site), researchers can implement quantitative habitat assessment protocols at Hellbender monitoring sites that will provide baseline data to assess the effect(s) of subsequent habitat changes on the population dynamics of Hellbenders. For these reasons, we strongly encourage state and federal agencies to adopt standardized methods to understand local and range-wide population dynamics and habitat associations of these unique aquatic salamanders.

The transect method yielded more Hellbender captures and greater CPUE compared to the conventional (2011) survey method. Moreover, using the transect method, we increased Hellbender captures at 66% of sites and increased CPUE at 83% of sites in comparison to surveys using the conventional timed-search method. Although this trend aligned with our hypothesis that the transect method would increase total captures and CPUE at occupied Hellbender sites, the observed increase in these metrics was not statistically significant. The lack of significant differences may have been due, in part, to our relatively small sample size (n = 6) and because the most dramatic increase in Hellbender captures and CPUE was observed at one densely populated site. This suggests that, by restricting search effort, we were less likely to overlook Hellbender cover rocks in each section. At another densely populated site, the number of total Hellbender captures decreased between 2011 and 2012 but overall CPUE increased. At sites with few Hellbender encounters (three or fewer), we also saw increases in Hellbender captures and CPUE at all but one site indicating that, perhaps, focusing search effort in discrete segments of habitat improves efficiency at low-density sites as well. Curiously, despite little variation in the number of Hellbender captures at sites between methods, CPUE increased at 83% of sites using the transect method suggesting that it improves survey efficiency at both low and high-density sites in comparison with the conventional timed-search method.

Researchers often use transects or quadrats in stream faunal surveys to standardize search area and increase the efficiency of survey efforts (Surber 1937; Cao et al. 2007; Crawford and Semlitsch 2007; Hart et al. 2016). Although incorporating transects may be beneficial to some study designs, transects may underestimate species diversity (compared with conventional timed searches) because they frequently, and by design, sub-sample populations from smaller areas (Samoilys and Carlos 2000; Smith 2006; Kadlec et al. 2012). Observed

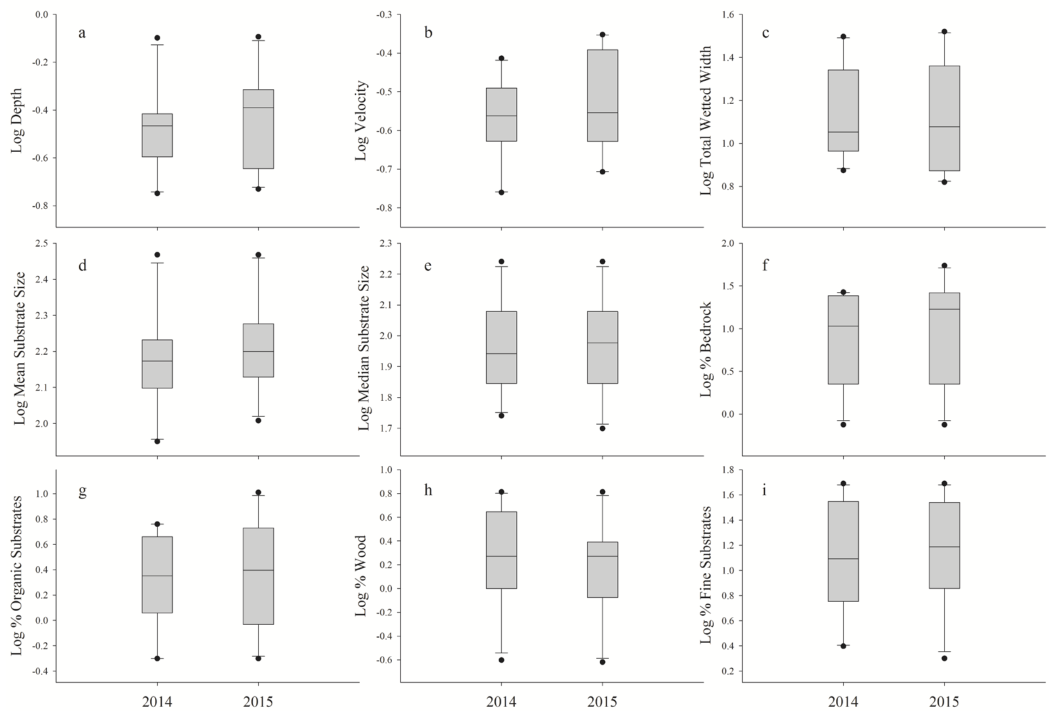

Figure 6. Comparison of physical habitat parameters from the New River Drainage between 2014 and 2015 field seasons. Boxes represent 25th and 75th percentiles and error bars represent 90th percentiles.

increases in capture rates and abundance estimates using our method are likely attributable to the fact that our transects encompassed the entire study reach and were not a sub-set as is typical with Surber or other quadrat-based sampling methods (Anderson et al. 1979). There is little investigation of the effectiveness of conventional timed searches versus transect methods where there is a single target species (Hart et al. 2016).

There is a possibility that Hellbenders migrated into study reaches between years; however, studies have shown that while Hellbenders move frequently, their home-ranges are usually small (Burgmeier et al. 2011) and there is little gene flow between meta-populations within river drainages (Unger et al. 2013). We also observed differences in some habitat parameters between years that might have influenced the number of captures and CPUE. Moreover, other factors (i.e., temperature, rainfall, poaching, etc.) that are variable among years may have influenced yields of our surveys. To compensate for this variability, we attempted to minimize year effects by using similar numbers of technicians, predominantly the same technicians, and we surveyed during the same time of year and under similar water clarity conditions (i.e., we did not conduct surveys in murky water).

Our analyses demonstrate that some habitat measurements (e.g., wetted width, depth) remain relatively consistent from year to year whereas others (e.g., substrate composition, current velocity) do not. Mountain streams are dynamic ecosystems and their physical habitat parameters are highly variable from year to year. Although this variability may potentially bias stream habitat measurements if comparisons are made without accounting for season or flow level (Poole et al. 1997), prior work using this method detected variation in habitat conditions that were associated with variability in Hellbender occupancy (Pugh et al. 2016). Moreover, although intra-class correlations revealed that measurements of some parameters between years were unrelated, we found substantial overlap of the 90% confidence intervals between years at most sites. This suggests that, although stream habitats are variable from year to year, this method may detect short-term (i.e., year to year) changes to physical habitat potentially resulting from droughts, changes to channel morphology, or increased inputs of fine substrates associated with land use change.

Although unstandardized, conventional timed searches may be valuable in previously un-surveyed streams, we argue that sampling a standardized length of stream reach and dividing reaches into equidistant transects has four important improvements over unstandardized conventional timed searches. First, investigators can more accurately quantify search time and effort; transects give searchers a chance to catch a break between searches to warm themselves, drink water or have a snack; and at localities where Hellbenders are abundant, smaller field teams (i.e., three to four technicians) must stop searching frequently so that researchers can process animals. During conventional surveys, it becomes difficult to keep track of search effort if the number of searchers changes during a survey; which frequently occurs as technicians leave the survey to process captured Hellbenders. Second, the transect-based approach seems to allow for more effective searching of available habitats. In reaches where searches are unconstrained, there is a greater potential for inaccurate estimates of search time and increased likelihood that technicians lose track of exact survey location (i.e., duplicating search effort where habitat has already been searched). Third, the transect framework allows for a better understanding of Hellbender microhabitat use and has potential to help researchers track the movement and habitat use of individual Hellbenders by using more precise measurement technologies (e.g., PIT tags and sub-meter GPS units). For example, the transect framework could be used to conduct a randomized experimental design using multiple transect numbers (1–15). Finally, this approach greatly facilitates quantifying stream physical habitat parameters and monitoring changes in Hellbender habitat quality. Implementation of this protocol would therefore enhance management practices by further elucidating threats to Hellbender habitat and standardizing efforts across the range allowing for collaboration in understanding regional changes in Hellbender habitat quality through meta-analyses.

Acknowledgments.—We thank the reviewers and editors for helpful comments and criticism. We thank Lori Williams and John Groves for technical support and advice and Lori Williams for reading an earlier version of this manuscript. We also thank the North Carolina Wildlife Resources Commission, Tennessee Wildlife Resources Agency, United States Forest Service, and National Park Service for permitting and for providing logistical support. Data were collected under permission of the Institutional Animal Care and Use Commission Committee at Appalachian State University (Protocol #10-14). Funding for this research was provided by the North Carolina Wildlife Resources Commission, North Carolina Zoological Park, Orianne Society, Foundation for the Conservation of Salamanders, Cryptobranchid Interest Group, and the Appalachian State University Department of Biology and Office of Student Research.

Literature Cited

Anderson, D.R., J.L. Laake, B.R. Crain, and K.P. Burnham. 1979. Guidelines for line transect sampling of biological populations. Journal of Wildlife Management 43:70–78.

Bodinof, C.M., J.T. Briggler, M.C. Duncan, J. Beringer, and J.J. Millspaugh. 2011. Historic occurrence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* in Hellbender *Cryptobranchus alleganiensis* populations from Missouri. Diseases of Aquatic Organisms 96:1–7.

Briggler, J.T., J. Utrup, C. Davidson, J. Humphries, J. Groves, T. Johnson, J. Ettling, M. Wanner, K. Traylor-Holzer, D. Reed, et al. 2007. Hellbender population and habitat viability assessment. International Union for Conservation of Nature/Species Survival Commission Conservation Breeding Specialist Group Report. International Union for Conservation of Nature, Gland, Switzerland. 118 p.

Brower, J.E., and J. H. Zar. 1998. Analysis of habitats. Pp. 25–51 *In* Field and Laboratory Methods for General Ecology. Wm. C. Brown Company Publishers, Dubuque, Iowa, USA.

Burgmeier, N.G., T.M. Sutton, and R.N. Williams. 2011. Spatial ecology of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Herpetologica 67:135–145.

Cao, Y., C.P. Hawkins, D.P. Larsen, and J. Van Sickle. 2007. Effects of sample standardization on mean species detectabilities and estimates of relative differences in species richness among assemblages. American Naturalist 170:381–395.

Crawford, J., and R.D. Semlitsch. 2007. Estimation of core terrestrial habitat for stream-breeding salamanders and delineation of riparian buffers for protection of biodiversity. Conservation Biology 21:152–158.

Dauwalter, D.C., and E.J. Pert. 2003. Electrofishing effort and fish species richness and relative abundance in Ozark Highland streams of Arkansas. North American Journal of Fisheries Management 23:1152–1166.

Foster, R.L., A.M. McMillan, and K.J. Roblee. 2009. Population status of Hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River Drainage of New York State. Journal of Herpetology 43:579–588.

Franklin, T.W. 2016. Estimates of Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) occupancy and detection using two sampling methods. M.S. Thesis, Appalachian State University, Boone, North Carolina, USA. 75 p.

Greene, B.T., W.H. Lowe, and G.E. Likens. 2008. Forest succession and prey availability influence the strength and scale of terrestrial-aquatic linkages in a headwater salamander system. Freshwater Biology 53:2234–2243.

Hart, M., C. Randklev, J. Dickson, N. Ford, B. Hernandez, and A. Schwalb. 2016. A literature review of freshwater mussel survey and relocation guidelines. Biological Report (0–6865), Texas Department of Transportation, Austin, Texas, USA. 47 p.

Hillis, R.E., and E.D. Bellis. 1971. Some aspects of the ecology of the Hellbender, *Cryptobranchus alleganiensis alleganiensis*, in a Pennsylvania stream. Journal of Herpetology 5:121–126.

Huang, J., Y. Cao, and K.S. Cummings. 2011. Assessing sampling adequacy of mussel diversity surveys in wadeable Illinois streams. Journal of the North American Benthological Society 30:923–934.

Kadlec, T., R. Tropek, and M. Konvicka. 2012. Timed surveys and transect walks as comparable methods for monitoring butterflies in small plots. Journal of Insect Conservation 16:275–280.

Maddock, I. 1999. The importance of physical habitat assessment for evaluating river health. Freshwater Biology 41:373–391.

Mayasich, J., D. Grandmaison, and C. Phillips. 2003. Eastern Hellbender status assessment report. Natural Resources Research Institute and Illinois Natural History Survey Report (NRRI/TR-2003/09), Champaign, Illinois, USA. 43 p.

McCluskey, S.M., and R.L. Lewison. 2008. Quantifying fishing effort: a synthesis of current methods and their applications. Fish and Fisheries 9:188–200.

Meador, M.R., J.P. McIntyre, and K.H. Pollock. 2003. Assessing the efficacy of single-pass backpack electrofishing to characterize fish community structure. Transactions of the American Fisheries Society 132:39–46.

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* Grobman. Applied Herpetology 4:207–216.

Nickerson, M.A., and C.E. Mays. 1973. The Hellbenders: North American "giant salamanders." Milwaukee Public Museum, Milwaukee Wisconsin, USA. 106 p.

Nickerson, M., and K.L. Krysko. 2003. Surveying for Hellbender salamanders, *Cryptobranchus alleganiensis* (Daudin): a review and critique. Applied Herpetology 1:37–44.

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

Pitt, A.L., J.L. Shinskie, J.J. Tavano, S.M. Hartzell, T. Delahunty, and S.F. Spear. 2017. Decline of a giant salamander assessed with historical records, environmental DNA and multi-scale habitat data. Freshwater Biology 2017:1–10.

Poole, G.C., C.A. Frissell, and S.C. Ralph. 1997. Instream habitat unit classification: inadequacies for monitoring and some consequences for management. Journal of The American Water Resources Association 33:879–896.

Pugh, M.W., J.D. Groves, L.A. Williams, and M.M. Gangloff. 2013. A previously undocumented locality of Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in the Elk River, Carter County, TN. Southeastern Naturalist 12:137–142.

Pugh, M.W., M. Hutchins, M. Madritch, L. Siefferman, and M.M. Gangloff. 2016. Land-use and local physical and chemical habitat parameters predict site occupancy by Hellbender salamanders. Hydrobiologia 770:105–116.

Quinn, S.A., J.P. Gibbs, M.H. Hall, and P.J. Petokas. 2013. Multiscale factors influencing distribution of the Eastern Hellbender salamander (*Cryptobranchus alleganiensis alleganiensis*) in the northern segment of its range. Journal of Herpetology 47:78–84.

Reimer, A., A. Mase, K. Mulvaney, N. Mullendore, R. Perry-Hill, and L. Prokopy. 2013. The impact of information and familiarity on public attitudes toward the Eastern Hellbender. Animal Conservation 2013:1–9.

Rödel, M.O., and R. Ernst. 2004. Measuring and monitoring amphibian diversity in tropical forests. I. An evaluation of methods with recommendations for standardization. Ecotropica 10:1–14.

Samoilys, M.A., and G. Carlos. 2000. Determining methods of underwater visual census for estimating the abundance of coral reef fishes. Environmental Biology of Fishes 57:289–304.

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

Smith, D.R. 2006. Survey design for detecting rare freshwater mussels. Journal of the North American Benthological Society 25:701–711.

Souza, M.J., M.J. Gray, P. Colclough, and D.L. Miller. 2012. Prevalence of infection by *Batrachochytrium dendrobatidis* and *Ranavirus* in Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in eastern Tennessee. Journal of Wildlife Diseases 48:560–566.

Surber, E.W. 1936. Rainbow Trout and bottom fauna production in one mile of stream. Transactions of the American Fisheries Society 66:193–202.

Unger, S.D., O.E. Rhodes, T.M. Sutton, and R.N. Williams. 2013. Population genetics of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) across multiple spatial scales. PLoS ONE, 8, 1-14. https://doi.org/10.1371/journal.pone.0074180.

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, L.A., and J.D. Groves. 2014. Prevalence of the amphibian pathogen *Batrachochytrium dendrobatidis* in Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) in Western North Carolina, USA. Herpetological Conservation and Biology 9:454–467.

Wolman, G.M. 1954. A method of sampling coarse river-bed material. Transactions of the American Geophysical Union 35:951–956.

Yoccoz, N.G., J.D. Nichols, and T. Boulinier. 2001. Monitoring of biological diversity in space and time. Trends in Ecology and Evolution 16:446–453.

M. Worth Pugh is the Coordinator of Zoological Collections for the Department of Biological Sciences at the University of Alabama in Tuscaloosa, USA. He received his M.S. in Biology from Appalachian State University (ASU), Boone, North Carolina, USA, in August 2013 and served as Collections Manager for the Department of Biology at ASU from 2014 to 2017. His thesis research focused on the impacts of land-use on Hellbender salamanders in northwestern North Carolina, USA. Worth currently works on a variety of projects involving freshwater mussels, fishes, and amphibians in the southeastern U.S. (Photographed by Mary Joan Pugh).

Thomas Franklin is the Environmental DNA Program Coordinator at the National Genomics Center for Wildlife and Fish Conservation, part of the Rocky Mountain Research Station of the U.S. Forest Service in Missoula, Montana, USA. He received his M.S. in Biology from Appalachian State University, Boone, North Carolina, USA, in May 2016 where his thesis research focused on comparing eDNA and traditional survey methods for estimating probabilities of occupancy and detection for Hellbender salamanders. Thomas currently designs and collaborates on a variety of eDNA based projects focusing on rare and invasive species across North America. (Photographed by U.S. Forest Service).

Lynn Siefferman is an Associate Professor of Biology at Appalachian State University (ASU), Boone, North Carolina, USA. She earned her Ph.D. from Auburn University, Auburn, Alabama, USA, studying the function and evolution of plumage coloration and personality in Eastern Bluebirds (Sialia sialis). Her current research interests include behavioral ecology and conservation biology. Dr. Siefferman teaches Ecology, Animal Behavior, and Ornithology lectures as well as graduate seminars at ASU. (Photographed by Appalachian State University).

Michael M. Gangloff is an Associate Professor of Biology at Appalachian State University (ASU), Boone, North Carolina, USA. He received his Ph.D. from Auburn University, Auburn, Alabama USA, in 2003. His research interests include population and community ecology, taxonomy, systematics, and the impacts of human-driven environmental changes on sensitive aquatic organisms. Dr. Gangloff teaches Conservation Biology, Aquatic Biology, Ecology, and graduate seminar courses at ASU. (Photographed by M. Worth Pugh).

Appendix 1. Comparison of habitat parameters in the Watauga Drainage between sampling years 2011 and 2012. All values are means excluding Median Substrate Size.

	Depth (m)		Velocity (m/s)		Substrate Size (mm)		Median Substrate Size (mm)		Total Wetted Width (m)		% Wood		% Bedrock		% Organic		% Boulder		% Fine	
Site	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
1	0.51	0.53	0.20	0.37	138	193	110	150	15.5	17.2	0.00	0.25	15.3	14.3	0.3	1.8	8.5	1.5	9.0	3.8
2	0.43	0.47	0.25	0.49	137	322	120	220	18.7	16.8	0.00	0.00	37.3	8.5	0.0	1.3	2.0	3.0	6.5	2.0
3	0.38	0.25	0.12	0.26	110	173	72	130	8.1	6.2	1.00	0.00	8.3	2.8	1.5	0.3	0.0	0.3	19.5	7.8
4	0.28	0.32	0.20	0.34	140	146	100	80	9.4	9.4	0.00	1.92	45.3	27.0	0.5	4.3	0.0	0.8	16.8	16.5
5	0.28	0.50	0.25	0.45	154	144	130	100	16.2	17.3	1.25	0.50	7.8	1.0	1.0	0.3	0.0	0.5	6.0	12.3
6	0.42	0.49	0.56	0.23	332	149	250	93	27.2	18.7	0.25	0.10	75.3	35.9	3.5	2.1	0.8	0.3	3.5	5.5
7	0.26	0.33	0.46	0.38	188	224	150	210	19.2	16.6	0.00	0.00	11.3	4.3	0.0	4.5	0.3	0.3	6.3	15.8
8	0.41	0.45	0.28	0.21	245	385	127.5	270	22.1	23.3	0.00	0.00	54.5	51.5	0.5	2.0	0.8	0.5	15.3	17.8
9	0.36	0.44	0.26	0.34	82	108	70	80	9.9	9.5	0.00	0.25	21.0	20.3	0.5	1.8	0.0	0.0	23.3	27.3
10	0.29	0.42	0.27	0.45	112	166	80	140	25.8	21.9	0.00	0.50	0.8	2.8	0.0	8.0	1.3	0.0	16.5	13.3
11	0.19	0.25	0.62	0.24	176	221	130	160	14.3	14.7	0.25	1.25	10.3	8.8	0.0	1.8	0.0	2.5	1.8	14.8
12	0.27	0.26	0.46	0.19	159	262	80	210	9.6	10.9	1.00	4.25	32.3	29.3	2.8	1.0	0.0	0.0	7.0	21.5
13	0.24	0.26	0.23	0.37	214	207	93.5	110	6.6	7.4	0.00	0.25	33.3	25.3	0.8	0.3	0.3	0.3	15.8	10.5
14	0.27	0.34	0.21	0.26	244	198	170	150	10.6	11.4	0.25	0.00	6.3	6.3	3.0	0.8	0.5	0.0	22.3	20.0
15	0.33	0.26	0.23	0.24	200	218	112.5	150	12.9	13.3	0.00	0.00	30.5	30.8	2.8	1.3	0.8	1.0	28.5	27.3
16	0.42	0.64	0.26	0.48	159	130	60	75	24.4	24.7	0.00	0.75	21.3	12.0	0.5	7.3	0.3	0.3	23.5	14.8
17	0.27	0.39	0.41	0.34	145	184	125	130	10.4	10.3	0.50	0.00	14.3	15.8	4.8	1.0	10.8	0.0	19.8	10.8
18	0.28	0.37	0.26	0.30	121	111	70	50	8.8	9.5	0.25	2.00	31.5	30.5	0.5	2.3	6.5	4.3	22.8	26.5
19	0.30	0.31	0.39	0.36	114	244	100	150	11.2	12.3	0.00	0.00	44.3	36.0	0.3	1.8	1.8	0.0	12.0	8.5
20	0.30	0.31	0.30	0.35	140	236	110	200	20.1	19.5	0.00	0.00	14.3	18.0	0.5	2.3	0.0	0.3	16.0	9.8

Appendix 2. Comparison of habitat parameters in the New Drainage between sampling years 2014 and 2015. All values are means excluding Median Substrate Size.

	Depth (m)		Velocity (m/s)		Substrate Size (mm)		Median Substrate Size (mm)		Total Wetted Width (m)		% Wood		% Bedrock		% Organic		% Boulder		% Fine	
Site	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
1	0.36	0.39	0.27	0.29	164	164	120	120	12.1	12.2	2	2.00	2.3	2.3	5.8	5.8	0.0	0.0	34.3	33.5
2	0.21	0.22	0.26	0.40	102	102	70	70	7.5	6.6	1.75	1.75	0.8	0.8	0.8	0.8	0.0	0.0	7.0	7.0
3	0.38	0.46	0.31	0.36	175	175	120	120	11.4	11.6	1.25	1.25	2.3	2.3	0.5	0.5	0.0	0.0	49.0	49.0
4	0.18	0.23	0.28	0.26	141	154	110	100	9.1	7.5	2.5	2.00	3.5	13.0	0.0	2.8	0.3	0.0	2.5	14.0
5	0.33	0.56	0.34	0.20	158	138	70	50	9.2	12.4	5	0.50	12.5	17.5	1.8	1.5	0.3	2.5	10.0	24.5
6	0.41	0.43	0.39	0.25	89	294	55	174	31.4	28.8	1	0.24	26.8	25.0	4.0	2.3	0.5	0.5	22.0	15.3
7	0.37	0.45	0.17	0.43	294	163	174	80	27.1	33.1	0.25	2.25	25.0	31.0	2.3	10.3	0.5	0.0	15.3	15.5
8	0.80	0.81	0.18	0.20	134	134	70	70	20.4	21.3	6.5	6.50	24.0	24.0	5.3	5.3	1.3	1.3	38.3	38.3
9	0.29	0.27	0.32	0.44	135	135	90	90	11.2	10.8	1	1.00	16.3	16.3	3.3	3.3	0.0	0.0	7.3	7.3
10	0.27	0.19	0.27	0.27	169	238	85	102	10.1	7.3	4.25	3.25	9.3	54.8	1.8	1.0	0.0	0.0	3.0	2.0