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Life-Stage Differences in Microhabitat Use by Hellbenders (*Cryptobranchus alleganiensis*)

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Abstract: Hellbenders (*Cryptobranchus alleganiensis*) are long-lived, fully aquatic salamanders that inhabit cool, well-oxygenated streams and rivers in the eastern United States. Although once abundant, *C. alleganiensis* populations have experienced major declines across the historical range. Habitat degradation, siltation, aquatic contaminants, and infectious diseases are commonly suggested as contributors to these declines. Although Tennessee provides areas of high-quality habitat for *C. alleganiensis*, microhabitat differences among life stages are not well documented. We evaluated microhabitat use of larval, subadult, and adult *C. alleganiensis* at three streams in east Tennessee by comparing sites occupied by *C. alleganiensis* to random sites within each stream. We used multivariate analysis to evaluate microhabitat use differences among larval, subadult, and adult *C. alleganiensis*. We completed habitat assessments for 60 individuals. We detected an association between *C. alleganiensis* presence (regardless of life stage) and the percentage of large rock, the percentage of low embedded rocks, and the number of rocks above 500 mm. Furthermore, the volume of cover rock, the number of rocks above 500 mm, the distance to bank, and the percentage of low embedded rocks, gravel, and sand were the most important microhabitat attributes to discriminate life-stage distributions. Overall, our analyses identify microhabitat attributes that are potentially important for long-term *C. alleganiensis* conservation and provide guidance for stream protection and restoration practices that might mitigate sedimentation and habitat degradation in impacted streams.

Key words: Cryptobranchid; Habitat use; Larval habitat use; Linear mixed models; Substrate requirements; Use versus availability

Amphibian population declines across the United States and worldwide have been linked to introduced species, overexploitation, habitat fragmentation, environmental contaminants, climate change, and infectious diseases (e.g., Blaustein et al. 1994; Lannoo 2005; Cushman 2006; Gallant et al. 2007; Collins and Crump 2009). Agriculture and landscape alteration (e.g., urbanization, construction of dams, and impoundments) represent primary forms of habitat degradation, and are leading threats to aquatic species that are either listed or proposed for listing under the Endangered Species Act (Wilcove et al. 1998; Malmqvist and Rundle 2002). In addition, urban development is often responsible for high rates of local extinction, loss of biodiversity, habitat homogenization, and replacement of native and rare species with nonnative species (McKinney 2002). As these threats increase in occurrence, anthropogenic disturbances will continue to represent one of the greatest challenges for future biodiversity conservation.

Land-altering practices have severely impacted habitat integrity of freshwater ecosystems (Malmqvist and Rundle 2002; Muenz et al. 2006; Henley et al. 2010). In the early 1990s, approximately 35% of freshwater amphibians and fishes, and 73% of freshwater mussels in North America were considered vulnerable, imperiled, or endangered on account of habitat degradation (Henley et al. 2010). Furthermore, extinction rates for mussels, crayfishes, fishes, and amphibians in North America could be five times higher than species losses in any terrestrial habitat (Ricciardi and Rasmussen 1999). This indicates that freshwater biodiversity faces threats on multiple levels (Lannoo 2005; Dudgeon et al. 2006).

Land alteration through intensive agriculture can cause physical changes in river and stream channels, disrupt flow, and disturb aquatic habitat through changes in chemical concentrations and sediment loads (Schultz et al. 1995; Malmqvist and Rundle 2002). Many agricultural practices can degrade riparian zones, which are critical for the control of sediment input, and maintenance of water quality and biotic integrity (Roth et al. 1996; Stevens and Cummins 1999). Sedimentation—the act of sediment filling interstitial spaces between rocks on the substrate of streams and rivers—facilitated through agricultural practices and deforestation, represents one of the major contributors to habitat degradation in freshwater systems, and has been linked to declines in amphibian populations (Welsh and Ollivier 1998; Muenz 2006; Barrett and Guyer 2008). Additionally, elevated rates of sedimentation impact community structure, disrupt local food webs, and negatively affect population demographics of local fauna and flora (Henley et al. 2010).

Hellbenders (*Cryptobranchus alleganiensis*) are fully aquatic salamanders in Family Cryptobranchidae that inhabit streams and rivers in the central and eastern United States. Cryptobranchids are represented globally by two genera and three species, and in North America are represented by Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) and Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*; Petranka 1998; Niemiller and Reynolds 2011). Hellbenders are habitat specialists and prefer cool, fast-flowing, well-oxygenated streams and rivers with a heterogeneous rock substrate (i.e., small, medium, and large sizes of rocks; Nickerson and Mays 1973; Humphries and Pauley 2005; Burgmeier et al. 2011a). Species presence has also been correlated with reduced amounts of organic and fine sediment (e.g., sand and silt), lower conductivity, and higher pH (Keitzer et al. 2013; Pugh et al. 2016; Pitt et al. 2017; Jachowski and Hopkins 2018). As a benthic species, *C. alleganiensis* use interstitial spaces between rocks within the stream substrate for shelter and reproductive sites (Nickerson and Mays 1973). Although once abundant, both *C. alleganiensis* subspecies have experienced major declines across their historical range (Williams et al. 1981; Wheeler et al. 2003; Foster et al. 2009).

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Impoundments, siltation, aquatic contaminants, and infectious diseases are suggested to be contributors to these declines (Nickerson and Mays 1973; Trauth et al. 1992; Nickerson et al. 2002; Burgmeier et al. 2011b). Because sedimentation decreases the availability of interstitial spaces between cover objects in the stream substrate, the process threatens the remaining *C. alleganiensis* populations across their range. Furthermore, *C. alleganiensis* have delayed sexual maturity (i.e., 5–8 yr for males), and likely have limited ability to withstand sustained high rates of larval mortality (Peterson et al. 1988; Wheeler et al. 2003). Therefore, to better protect this rare, long-lived species, it is important to understand how early life stages use instream habitat resources.

Despite over 30 yr of research conducted across the species range, most studies on habitat use have focused on general habitat requirements, status, and demographics of adult *C. alleganiensis*. Adult individuals are often easier to detect during surveys, whereas larval and subadult individuals are difficult to detect unless researchers use targeted survey techniques (Nickerson and Krysko 2003; Freake and DePerno 2017). Additionally, within declining populations where many studies have been conducted, adults are more abundant than other life stages (Foster et al. 2009). Because of the lack of research encompassing all *C. alleganiensis* life stages, researchers still do not fully understand *C. alleganiensis* larval ecology, habitat use and selection, and how larvae and subadults use available habitat compared to adults. Therefore, the purpose of our study was to (1) identify the microhabitat attributes that are associated with presence of adult, subadult, and larval *C. alleganiensis* within a stream section, and (2) evaluate differences in microhabitat use among adult, subadult, and larval *C. alleganiensis*. We expected to see ontogenetic shifts in microhabitat use, with larval *C. alleganiensis* using sites with low levels of fine sediment and high levels of gravel, pebble, and cobble, and adults using sites with comparatively larger, unembedded rocks.

Materials and Methods

Field Methods

Study area.—We conducted all surveys at streams located in eastern Tennessee, USA (Fig. 1). Our survey areas consisted of three delineated stream sections (i.e., generally 75 m in length) in three separate streams, and each habitat plot surveyed within a stream section was considered a site. We do not report exact stream locations because of conservation concerns, and to avoid illegal collection of animals and destruction of stream habitat. All three streams were located within public lands protected and managed by the National Park Service and US Forest Service in the Blue Ridge ecoregion of Tennessee. We selected these streams because previous survey work has indicated that all *C. alleganiensis* life stages were present at these streams (M. Freake, personal observation).

Snorkeling surveys and plot selection.—At each stream, prior to performing habitat surveys, we conducted snorkeling surveys where we lifted all rocks larger than 10 cm in diameter within a delineated stream section and searched for *C. alleganiensis* under cover objects. We returned all cover objects to the original locations to minimize habitat disturbance. We also recorded animals that were seen but not captured. For each individual captured, we recorded snout–vent length (mm), total length (mm), and mass (g), and marked each individual with a passive integrative transponder tag if individuals had not been previously marked. We released each individual at its site of capture. We used total length as a representation of life stage: individuals ≤125 mm were considered larval *C. alleganiensis*, individuals between 126 and 290 m were considered subadults, and individuals >290 mm were considered adults (Peterson et al. 1983; Jachowski 2016). We classified exact capture locations occupied by *C. alleganiensis* within the stream section as used sites. We selected unused sites based on random distances and directions within the delineated stream section, and assumed that these sites were not occupied. Random sites were located a maximum of 25 m up- or downstream from the used sites, so as to represent microhabitat that is available based on the spatial ecology reported by Burgmeier et al. (2011a).

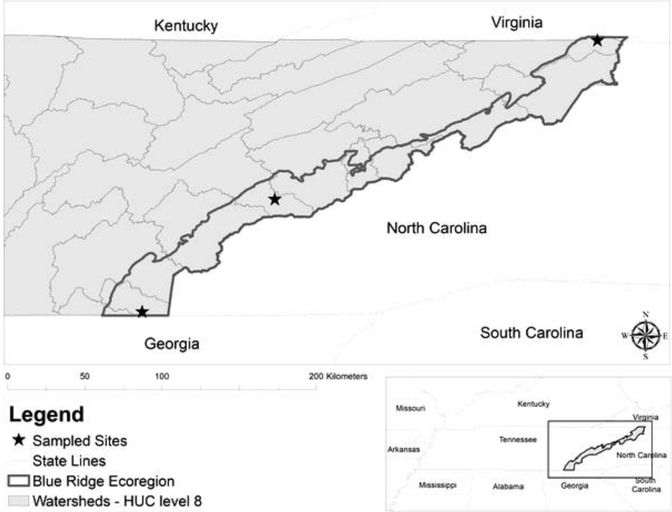

Fig. 1.—Location of stream sampling sites and subbasin watersheds representing medium-sized rivers (HUC8) in east Tennessee. The Blue Ridge ecoregion is indicated (bold lines) in both the large and small inset maps.

Microhabitat attribute characterization.—At both used and random sites, we categorized substrate into classes according to particle size and quantified the percentage of each substrate category within a quadrat following Welsh and Ollivier (1998; Table 1). We used a 0.75 × 0.75–m quadrat divided into 25 squares of equal size (i.e., each square represented 4% of the total 100%). At used sites, we placed the quadrat over the capture site and used the cover rock (i.e., rock used by the individual captured) as the plot center. At random sites, we placed the plot center over the substrate at the terminus of the random distance and bearing. We used the quadrat sampler to estimate the percentage of cover of the following substrate categories within each quadrat square: fine sediment (<0.06 mm), sand (0.06–2.0 mm), gravel (2.1–32.0 mm), pebble (32.1–64.0 mm), cobble (64.1–256.0 mm), large rock (>256.0 mm), bedrock, woody debris, vegetation, and algae (Table 1). To determine the total percentage of each substrate category within the quadrat, we estimated the percentage of each

TABLE 1.—Microhabitat attributes and stream characteristics measured during microhabitat surveys at three different *Cryptobranchus alleganiensis* streams in east Tennessee.

Microhabitat attribute	Code	Description
% fine sediment	fine_sediment	% fine sediment (<0.06 mm) within plot
% sand	sandy	% sand (0.06–2.0 mm) within plot
% gravel	gravel	% gravel (2.1–32.0 mm) within plot
% pebble	pebble	% pebble (32.1–64.0 mm) within plot
% cobble	cobble	% cobble (64.1–256.0 mm) within plot
% large rock	large_rock	% large rocks (>256.1 mm) within plot
% bedrock	bedrock	% bedrock within plot
% woody debris	woody_debris	% leaves of small woody debris within plot
% vegetation	vegetation	% underwater vegetation within plot
% algae	algae	% algae within plot
Volume of cover rock	cover_volume	Volume (cm^3) of cover rock on a used site or potential cover rock on a random site
No. of rocks above 500 mm	cover_above_500	No. of rocks larger than 500 mm in length
Low embeddedness	low_embed	% rocks larger than 256 mm with <50% of their surface embedded in the substrate
High embeddedness	high_embed	% rocks larger than 256 mm >50% of their surface embedded in the substrate
Stream characteristic	**Code**	**Description**
Stream width	stream_width	Stream width (m) measured at the center of the sampled site
Distance to bank	distance_to_bank	Distance (m) of cover rock to the nearest bank
Average water velocity	vel_average	Average water velocity (m^3/s)
Average water depth	wd_average	Average water depth (cm)

substrate category within each of the 25 quadrat squares and summed the percentage of each category over the 25 quadrat squares. Specific to the large rock category, we quantified the percentage of low embedded rocks (i.e., <50% of its surface embedded in the substrate) and high embedded rocks (i.e., >50% of its surface embedded in the substrate) by dividing the number of large rocks in each embeddedness category by the total number of large rocks within the quadrat. We determined the volume of the cover rock (i.e., the rock where an individual was located) at used sites. At random sites, we considered the largest rock closest to plot center as the cover rock. Lastly, we recorded the number of rocks larger than 500 mm in length as an additional means to evaluate the importance of rock size and quantity of large rocks for differentiating habitat use.

In addition to within-quadrat substrate measurements, we measured the following stream characteristics: stream width (m), distance of cover rock to bank (m), average water velocity (m^3/s), and average water depth (cm; Table 1). We used a standard measuring tape and a water velocity meter to measure water depth and water velocity at each side of the quadrat, respectively. Because water depth ranged widely between sampling locations, we measured water velocity in the lower 75% of the water column at each location when water depth permitted. We calculated the mean water depth and velocity from four measurements taken at each side of the quadrat.

Data Analyses: Among-Stream Analysis

Generalized Linear Mixed Model (GLMM).—As we did not find larval *C. alleganiensis* in Streams 2 and 3, we were unable to assess microhabitat use for this life stage among all study streams. Therefore, we merged larval and subadult age groups (hereinafter nonadults) to permit comparisons among study streams. We developed six a priori habitat models based on known *C. alleganiensis* habitat requirements and natural history (sedimentation, cover structure, location within stream, substrate, stream size, and life stage, as well as a global model; Table 2). We used a multiple hypothesis approach for data analysis because it is better suited for testing biologically relevant hypotheses, rather than a haphazard testing of all covariates simultaneously (Burnham and Anderson 2002). We used a GLMM with a binomial data distribution and logit link function via the lme4 package (Bates et al. 2015) in RStudio (RStudio Team 2015, Boston, MA) to evaluate how microhabitat features within each model correlated with presence of nonadult and adult *C. alleganiensis* at all three streams. As vegetation and algae were absent in all three streams, we did not include these attributes in our analysis. The sample sizes for percentages of fine sediment, woody debris, and bedrock were not adequate for analysis; therefore, we also removed these attributes from subsequent analyses.

Prior to analysis, we conducted a correlation analysis and standardized all data using z-scores.

The attributes of high and low percentages of rock embeddedness were highly correlated (correlation coefficient >0.70), so we chose to include low rock embeddedness only in our analysis (excluding high rock embeddedness to avoid overfitting of resulting models).

In this analysis, we considered use of habitat (i.e., used versus random sites) as the response variable, microhabitat attributes as fixed effects, and individual study stream as a random effect. The inclusion of individual study stream as a random effect is important to control for nonindependence of repeated microhabitat samples within a given stream. We used Akaike's information criterion (AIC) adjusted for small sample size (AIC_c; e.g., Burnham and Anderson 2002) to identify the model(s) that best explained differences between used and random sites for both adult and nonadult *C. alleganiensis*.

Probability of habitat use.—For both adults and nonadults, we used the Predict function in the lme4 package in RStudio (RStudio Team 2015) to determine probability of habitat use at incremental increases of microhabitat variables within top-ranked models (ΔAIC_c values <2.0) where confidence intervals of individual variables did not overlap zero. Lower and upper bounds of microhabitat variables modeled in probability plots were determined directly from data collected at our field sites.

TABLE 2.—Description and justification for habitat models used to evaluate the effect of different microhabitat attributes on the presence and absence of *Cryptobranchus alleganiensis* among three streams in east Tennessee. See Table 1 for a description of microhabitat attribute codes.

Model	Model terms	Justification
Sedimentation	low_embed + gravel + pebble	Sediment fills in interstitial spaces between substrate structures and can decrease microhabitat availability (Nickerson and Mays 1973; Nickerson et al. 2003).
Cover structure	cover_above_500 + large_rock + low_embed + cover_volume	Rock availability and interstitial spaces can determine shelter quality, along with breeding and nesting success (Keitzer et al. 2013; Quinn et al. 2013).
Location within stream	distance_to_bank + wd_average + vel_average	*Cryptobranchus alleganiensis* are not evenly distributed within a stream or river. For example, Burgmeier et al. (2011a) indicated that *C. alleganiensis* in Indiana use runs more often than pools and riffles.
Substrate	pebble + cobble + large_rock	Stream substrate can influence *C. alleganiensis* presence. Pugh et al. (2016), indicated that *C. alleganiensis* presence is correlated with greater particle size and bedrock, and reduced percentages of fine sediment.
Stream size	stream_width + vel_average + wd_average	Stream order and hydrological patterns can influence species diversity and distribution (Harrel et al. 1967; Platts 1979; Gordon et al. 2004).
Life stage	distance_to_bank + wd_average + cover_volume + gravel	Different *C. alleganiensis* size classes use different areas within the substrate. For example, larvae tend to prefer gravel substrate located closer to the stream edge (Nickerson et al. 2003).
Global	low_embed + gravel + cover_above_500 + large_rock + cover_volume + distance_to_bank + wd_average + vel_average + pebble + cobble + stream_width	Global model

Within-Stream Analysis

Discriminant Function Analysis (DFA).—Because Stream 1 was the only stream where we captured all *C. alleganiensis* life stages, (i.e., larval, subadult, and adult), we used DFA via SPSS (v24, IBM Corp, Armonk, NY) to further describe microhabitat differences among life stages within a single stream. DFA is often used to explain the difference or similarity among more than two well-defined naturally occurring groups (i.e., in our study, life stages) based on a set of explanatory parameters (McGarigal et al. 2000). DFA results are presented as multiple canonical functions that assess group membership according to the average of each variable. The first function maximizes differences between groups and denotes variables that have the highest contribution to those differences (Klecka 1980). Prior to analysis, we conducted a correlation and normality test. All microhabitat attributes besides percentage of large rock and average water depth were not normally distributed. Therefore, we performed a natural log transformation to satisfy normality requirements. We considered DFA results statistically significant when $P < 0.05$.

RESULTS

Capture Data

We sampled Stream 1 in 2015 and Streams 2 and 3 in 2016. We captured a total of 60 individuals: 35 individuals (9 larvae, 7 subadults, 19 adults) from Stream 1, 7 individuals (2 subadults, 5 adults) from Stream 2, and 11 individuals (3 subadults, 8 adults) from Stream 3. We observed an additional five adults on Stream 2 and two adults on Stream 3, but they were not captured because they were located under potential nest rocks (cf. Bishop 1941; Nickerson and Tohulka 1986) and we did not want to disturb a breeding location. We did not capture multiple individuals simultaneously or encounter individuals at random sites. We determined the sex of eight individuals (four females, four males) captured at Stream 1. Streams 2 and 3 were not sampled close to, or during breeding season (early to mid-September in these streams; M. Freake, personal observations) and animals did not display cloacal swelling, which is an external feature used to sex individuals.

Generalized Linear Mixed Model and Probability of Site Use

We sampled microhabitat at a total of 99 sites (60 used sites and 39 random sites). Overall, the cover structure model best explained adult *C. alleganiensis* presence among all three streams (model weight [ω_i] = 1.0; Table 3). Presence of adults was associated with percentage of large rock (large_rock, $\beta = 2.54 \pm 0.65$), followed by percentage of low embedded rocks (low_embed, $\beta = 1.95 \pm 0.55$), and the number of rocks larger than 500 mm within a quadrat (cover_above_500, $\beta = 0.75 \pm 0.36$; Table 4). The probability of microhabitat use by adult *C. alleganiensis* increased as large rock cover increased from 45% to 80%, low embedded rocks increased from 60% to 100%, and with presence of a minimum of three rocks larger than 500 mm (Figs. 2A–C, respectively). On average, adult Hellbenders used sites that had, on average, a greater number of rocks larger than 500 mm, more rocks with low embeddedness, and a greater percentage of large rock cover compared to nonadults (Table 5).

The cover structure model best explained nonadult *C. alleganiensis* presence among all three streams ($\omega_i = 0.98$; Table 3). Presence of nonadult *C. alleganiensis* was associated with percentage of large rock (large_rock, $\beta = –0.13 \pm 0.41$) and percentage of low embedded rocks (low_embed, $\beta = 1.95 \pm 0.65$; Table 4). The probability of microhabitat use by nonadults increased as large rock cover increased from 38% to 85% and low imbedded rocks increased from 55% to 100% (Figs. 3A,B, respectively). On average, nonadult subjects used sites that had higher percentages of sand, gravel, pebble, and cobble compared to sites used by adults (Table 5).

TABLE 3.—Results of predictive habitat models describing microhabitat attribute relationships among three streams in east Tennessee for larval, subadult, and adult *Cryptobranchus alleganiensis*. The log-likelihood (-LL), number of model parameters (K), Akaike's information criterion adjusted for small sample size (AIC_c) values, change in AIC_c (ΔAIC_c), and model weight (ω_i) are indicated below. See Table 2 for a description of microhabitat attribute codes and model justification.

	Adults					Nonadults				
Model	−LL	K	AIC_c	ΔAIC_c	ω_i	−LL	K	AIC_c	ΔAIC_c	ω_i
Sedimentation	42.18	5	95.20	32.20	0.00	31.74	5	74.59	18.72	0.00
Cover structure	24.91	6	63.00	0.00	1.00	21.15	6	55.88	0.00	0.98
Location within stream	52.61	5	116.05	53.05	0.00	35.74	5	82.58	26.71	0.00
Substrate	38.85	6	90.88	27.88	0.00	30.24	6	74.06	18.19	0.00
Stream size	52.87	5	116.57	53.56	0.00	34.15	5	79.41	23.54	0.00
Life stage	45.63	6	104.43	41.43	0.00	33.59	6	80.76	24.88	0.00
Global	23.48	13	78.66	15.65	0.00	14.71	13	63.32	7.45	0.02

Discriminant Function Analysis

Function 1 was the only significant function (Wilk's $\lambda = 0.125$, df $= 36$, $P < 0.001$) and explained 75.8% of the variance among life stages within Stream 1. Used microhabitat sites for subadults were most correctly classified (100%), followed by random sites (81.3%), sites used by adults (78.9%), and sites used by larvae (66.7%). Overall, 80.4% of original grouped cases were correctly classified. According to Function 1, volume of cover rock, number of rocks larger than 500 mm, distance to bank, and percentages of low embedded rocks, gravel, and sand were the most important microhabitat attributes to discriminate among life stages (Table 6). Our results indicated that the larval life stage was positively correlated with a greater percentage of gravel and sand, and greater distance to bank when compared to subadults and adults (Fig. 4). Similar to the GLMM results, sites used by larvae had, on average, lower percentages of large rock, volume of cover rock, and number of rocks larger than 500 mm compared to both subadults and adults (Table 7). Additionally, microhabitat use for both the subadult and adult life stages was positively associated with greater volume of cover rock, a greater number of rocks larger than 500 mm, and greater percentage of low embedded rocks (Fig. 4). Subadults and adults used sites that had on average lower percentages of gravel, pebble, and sand, and a greater percentage of cobble compared to sites used by larvae (Table 7). Sites used by larvae and random sites shared similar microhabitat attributes, whereas the sites used by other life stages did not share attributes with random sites (Fig. 4B).

DISCUSSION

Inadequate knowledge about habitat requirements of threatened and endangered species can limit the effectiveness of conservation and management efforts (Thompson 2004). Data on species' habitat requirements are essential for conservation at both the local (i.e., site mitigation and habitat restoration) and landscape (i.e., watershed and landscape conservation) scales. Our results indicate that within sampled streams, the percentage of large rock, availability of interstitial spaces under large rocks, and the number of rocks larger than 500 mm might be the most important limiting factors for the presence of all *C. alleganiensis* life stages. Pugh et al. (2016) and Humphries and Pauley (2005) have reported similar findings from streams in North Carolina and West Virginia, respectively. Our results are consistent with reports that *C. alleganiensis* life history is dependent on rock cover and interstitial spaces under rocks (Nickerson and Mays 1973; Peterson and Wilkinson 1996; Bodinof et al. 2012). It is important to note that, compared to adults, nonadults require a greater percentage of large rock cover to reach a similar probability of habitat use. This does not indicate that larval and subadult *C. alleganiensis* are using larger rocks compared to adults. We classified large rocks as any rock larger than 256 mm; which means that nonadults could be using sites with a greater percentage of large rocks, but not necessarily sheltering under rocks as large as those used by adults. Freake and DePerno (2017) demonstrated that, within an eastern Tennessee river, adult *C. alleganiensis* used rocks larger than nonadults, and that rocks used by adults averaged 100.9 cm in length. Because the number of rocks larger than 500 mcm in length was not a microhabitat attribute associated with presence of nonadults, we suggest that the optimal cover rock size for nonadults within our study streams likely falls between 256 and 500 mm.

Our results indicated a shift in microhabitat attributes used by different *C. alleganiensis* life stages and showed a distinction between used and random sites. Specifically, presence of *C. alleganiensis* larvae was positively correlated with greater percentages of gravel and sand, and greater distance to bank when compared to adults. We did not expect a positive relationship between larval presence and a greater percentage of sand because small particles fill in interstitial spaces between substrate structures and can

TABLE 4.—Beta coefficients, standard errors (SE), and 95% confidence intervals (CI) for all microhabitat attributes used in the "cover structure" model (Table 2) for both adult and nonadult *Cryptobranchus alleganiensis*. See Table 1 for a description of microhabitat attribute codes.

	Adults		Nonadults	
Model term	$\beta \pm$ SE	95% CI	$\beta \pm$ SE	95% CI
Cover_above_500	0.75 ± 0.36	0.04 to 1.46	−0.13 ± 0.41	−0.93 to 1.21
Large_rock	2.54 ± 0.65	1.27 to 3.81	2.86 ± 0.78	1.33 to 4.39
Low_embed	1.95 ± 0.55	0.87 to 3.03	1.95 ± 0.65	0.68 to 3.22
Cover_volume	−0.35 ± 0.40	−1.13 to 0.43	−0.61 ± 0.59	−1.77 to 0.55

TABLE 5.—Mean values (±1 SE) for microhabitat attributes at random sites and those used by adult and nonadult *Cryptobranchus alleganiensis* among three segments of three different streams in east Tennessee. "Used" and "random" columns represent average percentage of each substrate category within a 0.75-cm^2 quadrat and stream characteristics measured at each quadrat. Ranges of values are reported in parentheses (minimum–maximum); see Table 1 for a description of microhabitat attributes.

Microhabitat attribute	Random ($n = 39$)	Nonadults used ($n = 21$)	Adults used ($n = 39$)
% sand	3.3 ± 1.4 (0–39)	4.6 ± 1.5 (0–25)	1.5 ± 0.7 (0–20)
% gravel	6.5 ± 2.4 (0–67)	3.8 ± 1.4 (0–19)	2.9 ± 1.1 (0–29)
% pebble	21.5 ± 3.7 (0–81)	12.0 ± 2.8 (0–58)	11.1 ± 1.8 (0–44)
% cobble	21.2 ± 2.7 (0–65)	18.7 ± 2.7 (0–52)	16.5 ± 2.1 (0–43)
% large rock	32.3 ± 4.3 (0–94)	59.2 ± 3.5 (32–92)	63.8 ± 2.9 (30–100)
Volume of cover rock (cm^3)	46.1 ± 26.6 (0–945)	37.8 ± 8.9 (0.2–163.7)	82.6 ± 14.1 (5.28–364)
No. of rocks larger than 500 mm	0.7 ± 0.2 (0–4)	0.8 ± 0.1 (0–2)	1.5 ± 0.2 (0–6)
% low embeddedness	42.9 ± 0.1 (0–100)	67.0 ± 0.0 (0.3–100)	74.8 ± 0.0 (0–100)
Stream width (m)	15.1 ± 1.3 (5.4–35.7)	20.1 ± 1.9 (8.3–35.7)	16.8 ± 1.5 (1.7–35.7)
Distance to bank (m)	5.3 ± 0.7 (0–16.6)	6.4 ± 1.0 (1.3–18.2)	4.8 ± 0.5 (1.25–12.2)
Average water velocity (m^3/s)	1.0 ± 0.1 (0.1–3.5)	1.1 ± 0.2 (0.1–2.9)	1.0 ± 0.1 (0.1–2.9)
Average water depth (cm)	48.9 ± 4.7 (9.5–119.5)	66.7 ± 4.9 (28.2–108)	56.9 ± 5.0 (0.17–143.7)

decrease microhabitat availability for larvae (Nickerson and Mays 1973; Nickerson et al. 2003). As the percentage of gravel and sand cover decreased, and volume of rock cover, number of rocks larger than 500 mm, and percentage of low embedded rocks increased, the microhabitat characteristics transitioned from larval to subadult and adult habitat. Similar results have been reported by Keitzer et al. (2013), where abundant gravel substrate had a negative impact on microhabitat use of adult *C. alleganiensis* in West Virginia. This relationship could be influenced by the fact that our habitat sampling accounts for only the substrate that is on top of the stream or river bed. Therefore, all the gravel that is present under large rock is not accounted for, but might still be used as habitat by subadult and adult *C. alleganiensis*. Additionally, the parent substrate at our sampled streams is composed primarily of metamorphic sandstone and siltstone rocks, which produce greater pebble and cobblestone cover instead of gravel (Freake and DePerno 2017). The overlaps between the centroids representing the random sites and larval sites indicate that our analyses do not perfectly explain all differences in habitat use. Furthermore, we assigned subjects to each life stage according to predetermined sizes, and there is likely some overlap in habitat use occurring among individuals of similar sizes.

As anthropogenic changes continue to impact freshwater ecosystems and respective biodiversity, knowledge of *C. alleganiensis* microhabitat use is essential for conservation efforts (Strayer and Dudgeon 2010). Our study streams were selected for the purpose of evaluating microhabitat use differences among larval, subadult, and adult *C. alleganiensis*. Because streams that support populations that contain all *C. alleganiensis* life stages are limited in Tennessee (especially for larval stages), and are primarily restricted to the Blue Ridge ecoregion of east Tennessee, we did not establish study sites in additional ecoregions. Although our study quantified microhabitat use for a geographically restricted sample of *C. alleganiensis*, our data can be used as a starting point for restoration of degraded stream habitats when conservation, translocation, and repatriation efforts of captive-reared *C. alleganiensis* are undertaken. Because our results are limited to microhabitat use in high-quality stream systems, future research can increase inference by completing habitat sampling transects at discrete distances along sections of streams where *C. alleganiensis* are declining or are no longer present. Microhabitat sampling should be

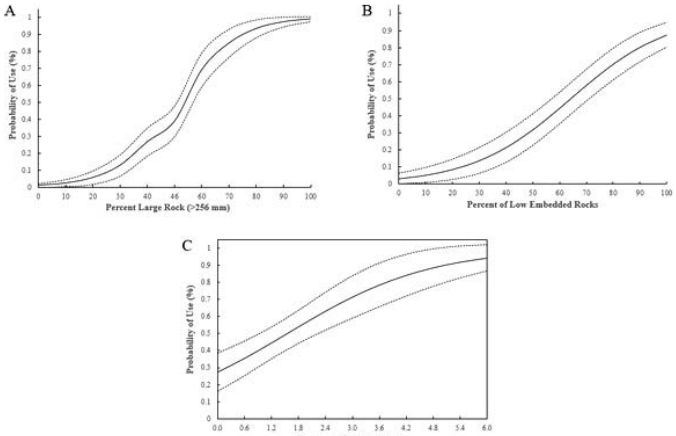

FIG. 2.—The effect of percentage of large rock (rocks >256 mm; A), percentage of low embedded rocks (i.e., rocks larger than 256 mm with <50% of their surface embedded in the substrate; B), and number of rocks >500 mm (C) on probability of site use by adult *Cryptobranchus alleganiensis* across all three streams. These microhabitat attributes were included in the top-ranked generalized linear mixed model microhabitat model (cover structure model), which best explained presence of adults. Dashed lines represent standard errors. When generating predictive plots, we held the mean constant for covariates that were not manipulated.

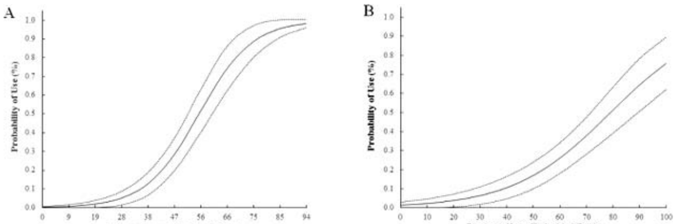

FIG. 3.—The effect of percentage of large rock (A), and percentage of low embedded rocks (i.e., rocks larger than 256 mm with <50% of their surface embedded in the substrate; B) on the probability of microhabitat use by nonadult (i.e., larval and subadult) *Cryptobranchus alleganiensis* across all three streams. These microhabitat attributes were included in the top-ranked generalized linear mixed model microhabitat model (cover structure model), which best explained presence of nonadults. Dashed lines represent standard errors. When generating predictive plots, we held the mean constant for covariates that were not manipulated.

Table 6.—Standardized canonical discriminant function coefficients for the first two discriminant functions of microhabitat attributes that discriminate *Cryptobranchus alleganiensis* life stage (larval, subadult, and adult) distribution within Stream 1. Values in bold have the highest weight within each function and were used to produce Fig. 4A,B. See Table 1 for a description of microhabitat attribute codes.

Microhabitat attribute	Function 1	Function 2
% sand	**−0.313**	0.370
% gravel	**−0.532**	**0.705**
% pebble	−0.134	**−0.442**
% cobble	−0.023	0.135
Stream width (m)	−0.037	**1.143**
Distance to bank (m)	**−0.499**	0.076
Average water velocity (m^3/s)	0.235	−0.031
Volume of cover rock (cm^3)	**0.798**	−0.244
% low embeddedness	**0.254**	0.155
No. of rocks larger than 500 mm	**0.348**	**0.446**
% large rock	−0.106	0.227
Average water velocity (m^3/s)	0.195	0.068

completed across a gradient of stream disturbance, and the resulting data paired with capture history and demographic data to better define the thresholds where in-stream microhabitat is no longer sufficient to provide required features to support the persistence of all *C. alleganiensis* life stages.

Prior to implementing local-scale conservation strategies (e.g., translocation target sites, stream restoration), we suggest evaluating stream microhabitat attributes, especially large rock availability and overall rock embeddedness. Furthermore, the restoration efforts should focus on the control or reduction of fine sediment that might potentially fill interstitial spaces between small substrate particles. Long-term solutions to reduce excess sediment likely depend on catchment-level sediment control through improved riparian habitat, instead of single-reach restoration efforts. Also, because our analyses were limited to the microhabitat attributes bound by a 0.75-m^2 quadrat, restoration efforts should include target placement of optimal microhabitat across a stream segment. For example, specific quadrats within a stream segment could be selected for improvement based on the requirements of each life stage, and the overall project goal. Overall, we suggest that areas of optimal habitat for adults consist of at least 45% large rock cover, a minimum of two rocks larger than 500 mm, and a minimum of 60% low embedded rocks. Subadult optimal habitat in these streams consists of large rocks between 256 and 500 mm, with at least 55% low embedded rocks. Optimal habitat for larval *C. alleganiensis* in eastern Tennessee consists of a greater percentage of gravel and sand, with some large rock cover, all located farther from the bank when compared to sites designated for subadult and adults. Developing small areas of optimal habitat within stream restoration sites might provide the baseline microhabitat attributes required to support local conservation efforts for Hellbenders.

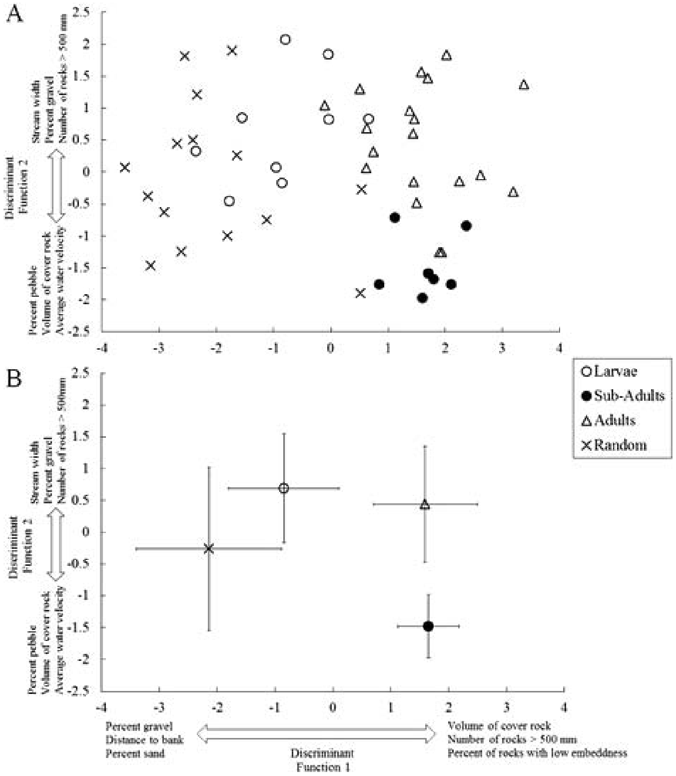

Fig. 4.—Discriminant function analysis diagram illustrating differences in distribution patterns among all life stages and random sites according to different microhabitat attributes (A), and their respective centroids (B). Overlapping groups share attributes, whereas groups that do not overlap do not share attributes. Error bars indicate ±1 SE.

Table 7.—Mean values (±1 SE) for microhabitat attributes at random sites and those used by larval, subadult, and adult *Cryptobranchus alleganiensis* within an east Tennessee stream that had all life stages represented (Stream 1). "Used" and "random" columns represent mean percentages of each substrate category within a 0.75-cm^2 quadrat and stream characteristics measured at each quadrat. Ranges of values are reported in parentheses (minimum–maximum); see Table 1 for a description of microhabitat attributes.

Microhabitat attribute	Random ($n = 16$)	Larva used ($n = 9$)	Subadult used ($n = 7$)	Adult used ($n = 19$)
% sand	2.8 ± 1.7 (0–25)	4.7 ± 2.8 (0–25)	1.0 ± 0.7 (0–5)	2.0 ± 1.2 (0–20)
% gravel	15.9 ± 4.9 (0–67)	6.9 ± 2.8 (0–19)	2.4 ± 1.4 (0–10)	5.9 ± 2.0 (0–29)
% pebble	15.9 ± 2.6 (0–32)	13.6 ± 6.3 (0–58)	11.4 ± 2.7 (6–26)	6 ± 1.6 (0–21)
% cobble	25.6 ± 5.0 (0–65)	12.7 ± 2.1 (5–22)	15.1 ± 3.6 (0–29)	15.4 ± 2.9 (0–43)
% large rock	34.6 ± 6.0 (0–94)	61.1 ± 5.5 (34–92)	66.1 ± 5.4 (52–92)	68.6 ± 4.2 (30–100)
Volume of cover rock (cm^3)	6.4 ± 3.3 (0–48.4)	21.5 ± 9.3 (0.2–82)	73.7 ± 17.5 (27.7–163.7)	97.6 ± 19.8 (21.4–318.5)
No. of rocks larger than 500 mm	0.6 ± 0.26 (0–3)	0.6 ± 0.2 (0–2)	1 ± 0.0 (0)	2 ± 0.3 (1–6)
% low embeddedness	47.6 ± 0.1 (0–100)	58.9 ± 0.1 (36.4–83.3)	76.7 ± 0.1 (33.3–100)	71.7 ± 0.0 (25–100)
Stream width (m)	23.1 ± 1.8 (14.1–35.7)	27.2 ± 2.2 (18.7–35.7)	18.9 ± .8 (14.1–24.5)	6.7 ± 1.7 (14.1–35.7)
Distance to bank (m)	8.0 ± 1.0 (3.62–16.6)	9.1 ± 1.8 (1.3–18.2)	5.4 ± 1.1 (2.65–11.2)	6.7 ± 0.7 (1.5–12.25)
Average water velocity (m^3/s)	1.4 ± 0.2 (0.5–3.5)	1.3 ± 0.2 (0.4–2.3)	1.2 ± 0.3 (0.5–2.9)	1.3 ± 0.2 (0.1–2.9)
Average water depth (cm)	58.4 ± 6.5 (9.7–98)	62.8 ± 5.7 (37.5–85.5)	80.7 ± 7.1 (52–108)	61.1 ± 6.4 (21.4–135)

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Literature Cited

Barrett, K., and C. Guyer. 2008. Differential responses of amphibians and reptiles in riparian and stream habitats to land use disturbances in western Georgia, USA. Biological Conservation 141:2290–2300.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48. DOI: https://dx.doi.org/10.18637/jss.v067.i01.

Bishop, S.C. 1941. The Salamanders of New York. New York State Museum Bulletin, USA.

Blaustein, A.R., D.B. Wake, and W.P. Sousa. 1994. Amphibian declines: Judging stability, persistence, and susceptibility of populations to local and global extinctions. Conservation Biology 8:60–71.

Bodinof, C.M., J.T. Briggler, R.E. Junge, J. Beringer, M.D. Wanner, C.D. Schuette, and J.J. Millspaugh. 2012. Postrelease movements of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*). Herpetologica 68:160–173.

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

Burgmeier, N.G., S.D. Unger, T.M. Sutton, and R.N. Williams. 2011b. Population status of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. Journal of Herpetology 45:195–201.

Burnham, K.P., and D. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer Science and Business Media, Germany.

Collins, J.P., and M.L. Crump. 2009. Extinction in Our Times: Global Amphibian Decline. Oxford University Press, UK.

Cushman, S.A. 2006. Effects of habitat loss and fragmentation on amphibians: A review and prospectus. Biological Conservation 128:231–240.

Dudgeon, D., A.H. Arthington, M.O. Gessner, Z.I. Kawabata, D.J. Knowler, C. Lévêque, and C.A. Sullivan. 2006. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews 81:163–182.

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.

Freake, M.J., and C.S. DePerno. 2017. Importance of demographic surveys and public lands for the conservation of eastern hellbenders *Cryptobranchus alleganiensis alleganiensis* in southeast USA. PLoS ONE 12:e0179153. DOI: https://doi.org/10.1371/journal.pone.0179153

Gallant, A.L., R.W. Klaver, G.S. Casper, and M.J. Lannoo. 2007. Global rates of habitat loss and implications for amphibian conservation. Copeia 2007:967–979.

Gordon, N.D., T.A. McMahon, and B.L. Finlayson. 2004. Stream Hydrology: An Introduction for Ecologists. John Wiley and Sons, USA.

Harrel, R.C., B.J. Davis, and T.C. Dorris. 1967. Stream order and species diversity of fishes in an intermittent Oklahoma stream. American Midland Naturalist 428–436.

Henley, W.F., M.A. Patterson, R.J. Neves, and A.D. Lemly. 2010. Effects of sedimentation and turbidity on lotic food webs: A concise review for natural resource managers. Reviews in Fisheries Science 8:125–139.

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.

Jachowski, C.M.B. 2016. Effects of Land Use on Hellbenders (*Cryptobranchus alleganiensis*) at Multiple Levels and Efficacy of Artificial Shelters as a Monitoring Tool. Ph.D. dissertation, Virginia Polytechnic Institute and State University, USA.

Jachowski, C.M.B., and W.A. Hopkins. 2018. Loss of catchment-wide riparian forest cover is associated with reduced recruitment in a long-lived amphibian. Biological Conservation 220:215–227.

Keitzer, S.C., T.K. Pauley, and C.L. Burcher. 2013. Stream characteristics associated with site occupancy by the eastern hellbender, *Cryptobranchus alleganiensis alleganiensis*, in southern West Virginia. Northeastern Naturalist 20:666–677.

Klecka, W.R. 1980. Discriminant Analysis. SAGE Publications, USA.

Lannoo, M.J. (ed.). 2005. Amphibian Declines: The Conservation Status of United States Species. University of California Press, USA.

Malmqvist, B., and S. Rundle. 2002. Threats to the running water ecosystems of the world. Environmental Conservation 29:134–153.

McGarigal, K., S.G. Stafford, and S. Cushman. 2000. Multivariate Statistics for Wildlife and Ecology Research. Springer, USA.

McKinney, M.L. 2002. Urbanization, biodiversity, and conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. BioScience 52:883–890.

Muenz, T.K., S.W. Golladay, G. Vellidis, and L.L. Smith. 2006. Stream buffer effectiveness in an agriculturally influenced area, southwestern Georgia. Journal of Environmental Quality 35:1924–1938.

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

Nickerson, M.A., and C.E. Mays. 1973. The Hellbenders: North American "Giant Salamanders." Milwaukee Public Museum, USA.

Nickerson, M.A., and M.D. Tohulka. 1986. The nests and nest site selection by Ozark Hellbenders, *Cryptobranchus alleganiensis bishopi* Grobman. Transactions of the Kansas Academy of Science 89:66–69.

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., K.L. Krysko, and R.D. Owen. 2003. Habitat differences affecting life stage distributions of the hellbender salamander, *Cryptobranchus alleganiensis*. Southeastern Naturalist 2:619–629.

Niemiller, M.L., and R.G. Reynolds (eds.). 2011. The Amphibians of Tennessee. University of Tennessee Press, USA.

Peterson, C.L., and R.F. Wilkinson. 1996. Home range size of the hellbender (*Cryptobranchus alleganiensis*) in Missouri. Herpetological Review 27:126–127.

Peterson, C.L., R.F. Wilkinson, Jr., M.S. Topping, and D.E. Metter. 1983. Age and growth of the Ozark hellbender (*Cryptobranchus alleganiensis bishopi*). Copeia 1983:225–231.

Peterson, C.L., Metter, B.T. Miller, R.F. Wilkinson, and M.S. Topping. 1988. Demography of the Hellbender *Cryptobranchus alleganiensis* in the Ozarks. American Midland Naturalist 119:291–303.

Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, 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 62:967–976.

Platts, W.S. 1979. Relationships among stream order, fish populations, and aquatic geomorphology in an Idaho river drainage. Fisheries 4:5–9.

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.

Ricciardi, A., and J.B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13:1220–1222.

Roth, N.E., J.D. Allan, and D.L. Erickson. 1996. Landscape influences on stream biotic integrity assessed at multiple spatial scales. Landscape Ecology 11:141–156.

Schultz, R.C., J.P. Collettil, T.M. Isenhart, W.W. Simpkins, C.W. Mize, and M.L. Thompson. 1995. Design and placement of a multi-species riparian buffer strip system. Agroforestry Systems 29:201–226.

Stevens, M.H.H., and K.W. Cummins. 1999. Effects of long-term disturbance on riparian vegetation and in-stream characteristics. Journal of Freshwater Ecology 14:1–17.

Strayer, D.L., and D. Dudgeon. 2010. Freshwater biodiversity conservation: Recent progress and future challenges. Journal of the North American Benthological Society 29:344–358.

Thompson, W.L. (ed.). 2004. Sampling Rare or Elusive Species: Concepts, Designs, and Techniques for Estimating Population Parameters. Island Press, USA.

Trauth, S.E., J.D. Wilhide, and R. Daniel. 1992. Status of the Ozark Hellbender, *Cryptobranchus bishopi* (Urodela: Cryptobranchidae), in the Spring River, Fulton County, Arkansas. Proceedings Arkansas Academy of Science 46:83–86.

Welsh, H.H., and L.M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: A case study from California's redwoods. Ecological Applications 8:1118–1132.

Wheeler, B.A., E.A. 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.

Wilcove, D.S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48:607–615.

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 9:94–99.

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