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PRIMARY RESEARCH PAPER

Land-use and local physical and chemical habitat parameters predict site occupancy by hellbender salamanders

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Abstract Anthropogenic landscapes negatively impact stream habitats by altering hydrologic, sediment, and nutrient cycling regimes, thereby reducing or displacing populations of sensitive biota. The hellbender (*Cryptobranchus alleganiensis*) is an imperiled salamander endemic to eastern and central North American streams. Although once widespread, hellbender distributions have contracted and populations have declined in the past several decades. Hellbenders are considered indicators of stream quality; however, few studies have empirically linked hellbender presence to stream habitat or water-quality. We examined the ability of catchment-scale land-use and local physical and chemical habitat parameters to predict hellbender occurrence in an Appalachian headwater river drainage. Generalized linear models revealed that water-quality, local habitat, and catchment land-use are informative predictors of hellbender site occupancy. Because broad-scale land-use changes likely affect hellbender populations at multiple levels, management and conservation should focus on protecting streams at the catchment scale. In this system, ex-urban development appears to be the primary threat to hellbenders. However, threats to hellbender populations may be mitigated by management regulations targeting economically important outdoor recreational activities including trout fishing as well as existing streamside development guidelines.

Keywords *Cryptobranchus alleganiensis* · Appalachian Mountains · Generalized linear model · Stream quality · Indicator species

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Introduction

Current and historical land-use may greatly affect stream ecosystem function and integrity (Huston, 2005; Moore & Palmer, 2005; Krause et al., 2008; Maloney et al., 2008). The frequency and intensity of disturbance resulting from land-use change often reduces stream water-quality, invertebrate and fish diversity, and ultimately, ecosystem services. Land-use change may also intensify the effects of hydrologic events resulting in substrate heterogeneity reduction as well as increased nutrient and sediment inputs (see Paul & Meyer, 2001; Allan, 2004; Barrett & Guyer, 2008; Carpenter et al., 2011; Maloney & Weller, 2011). Altered physical and chemical conditions may also negatively impact populations of sensitive lotic taxa including benthic insects, mollusks, fishes, and amphibians. Generally, it is understood that local species richness in these taxa is positively related to the stability and integrity of nutrient cycling, flow regimes, and substrate composition. Increased sediment loads and decreased substrate heterogeneity may reduce shelter and survivorship of interstitial organisms and have negative effects on other important components in stream ecosystems (see Collares-Pereira & Cowx, 2004; Dudgeon et al., 2006; Stendera et al., 2012). Therefore, consideration of these impacts is important to creating and implementing effective water-quality and wildlife conservation management.

Numerous studies have examined land-use effects on stream amphibian populations and community composition (see Collins & Storfer, 2003; Beebee & Griffiths, 2005). Studies of headwater streams indicate that increases in land-use disturbance reduce abundance, species richness, and body condition of aquatic amphibians (Freda, 1986; Welsh & Ollivier, 1998; Cushman, 2006; Barrett & Price, 2014). Aquatic and semi-aquatic salamanders are considered indicator species in stream ecosystems because they are long-lived while concomitantly having physiologies that are largely open to the environment (Duellman & Trueb, 1985; Welsh & Ollivier, 1998). Many aquatic salamanders are regional endemics and are likely adapted to specific local environmental conditions (see Petranka, 1998). Thus, management strategies should consider land-use change as a threat to aquatic salamander diversity.

Hellbenders (*Cryptobranchus alleganiensis*; Daudin) are large salamanders (>40 cm total length) that are fully aquatic and endemic to upland streams of the Appalachian Mountains and Ozark Highlands (Smith, 1907; Nickerson & Mays, 1973; Petranka, 1998). Currently, there are two sub-species recognized: the eastern hellbender (*C. a. alleganiensis*) and the Ozark hellbender, *C. a. bishopi,* (Nickerson & Mays, 1973); however phylogenetic analyses suggest these taxon designations are problematic and may be artificial (Routman et al., 1994; Sabatino & Routman, 2009; Tonione et al., 2011). Hellbenders are long-lived and require specific habitat and prey items to maintain viable populations. Although considered indicators of high water-quality (Hillis & Bellis, 1971; Nickerson & Mays, 1973; Nickerson et al., 2003), few studies have quantified habitat, water-quality, and land-use effects on hellbender populations or empirically linked these parameters to hellbender occurrence (Keitzer et al., 2013; Quinn et al., 2013).

Recent studies suggest that hellbender populations are declining across their range (Mayasich et al., 2003; Briggler et al., 2007b). There are several plausible causes of range-wide hellbender declines. However, recent changes to local and regional land-use in the Southern Appalachian Mountains (the range core for hellbenders) including ex-urban development in formerly rural regions has most frequently been linked to broad-scale changes to stream habitats. Increasing levels of fine sediments and nutrients associated with recent development in this region may reduce interstitial space available to hellbenders for shelter and oviposition and increased hydrologic variability may increase stresses associated with drought and high-water periods (O'Driscoll et al., 2010). Land-use change has also been shown to have temporarily persistent influence on populations of other lotic salamanders in this region and it seems reasonable to assume that hellbenders are similarly affected by changes to land-use that occurred prior to the passage of the Clean Water or Endangered Species Acts (Surasinghe & Baldwin, 2014, 2015).

Here, we quantified hellbender occurrence at both local (e.g., reach-scale) physical and chemical parameters and landscape (e.g., land-use/land-cover) scales in a forested and relatively undeveloped river drainage in western NC and eastern TN to determine whether habitat parameters predict hellbender occurrence. We predicted that hellbenders would occur in localities that exhibited higher water-quality, heterogeneous substrate composition, and less developed upstream land-use and land-cover.

Methods

Hellbender surveys

We sampled hellbenders during June–August from 2010 to 2013 at 21 sites in a headwater drainage in western North Carolina (Fig. 1). All sites consisted of a 150-m stream reach divided by cross-channel transects at 10-m intervals ($n = 16$ per site). Sites were selected based on occurrence data from historical records and accessibility (i.e., less logistic difficulty in accessing sites). We scouted all sites prior to sampling to ensure that they contained suitable hellbender habitat (i.e., large to medium sized rocks, deep pools, fast-flowing riffles; Hillis & Bellis, 1971; Nickerson & Mays, 1973).

We used timed visual-tactile surveys and snorkeled in an upstream direction while systematically turning rocks by hand or by using log peaveys and captured hellbenders by hand or allowed them to drift into dip nets (Nickerson & Krysko, 2003). While some have hypothesized that these surveys do not target larval habitat (Nickerson & Krysko, 2003; Foster et al., 2009), recent research suggests that, in some cases, larvae are found more often in cobble and boulder habitat than in smaller gravel habitat (Hecht-Kardasz et al., 2012). If visibility appeared impaired due to turbulent water conditions caused by large rain events, we did not conduct surveys to minimize the probability of missing an animal in murky water. We calculated catch per unit effort (CPUE) as the number of hellbenders captured per person hour (number of people searching × search time) at the site scale. We classified sites as "present" if a hellbender was detected at least one time in a site and classified a site as "absent" if a hellbender was not detected in a site through the duration of the study. All sites were searched 2–4 times between 2010 and 2013 depending on the site (Supplementary Material 1). Detection probability (P) was >0.7 meaning that two surveys at each site satisfies the minimum requirement for confidently classifying "absent" sites (MacKenzie &

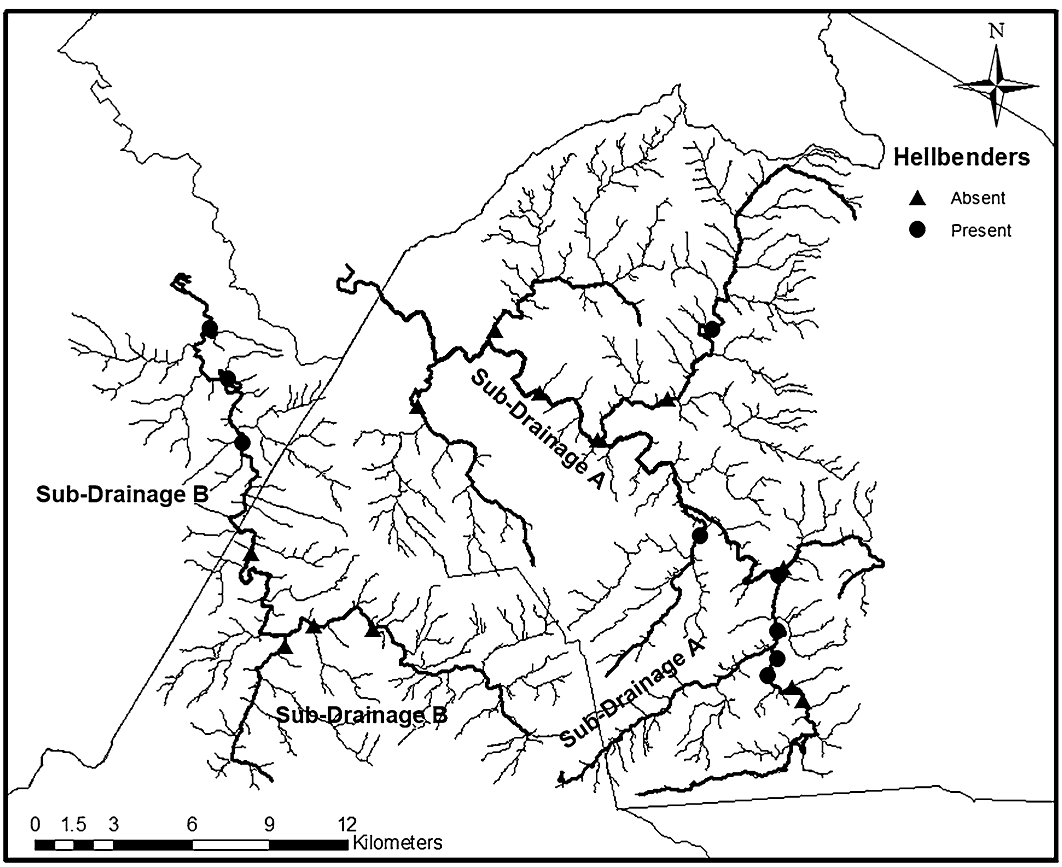

Fig. 1 Map of study streams and site localities in the Watauga River Drainage in northwestern North Carolina and northeastern Tennessee. Sites where hellbenders were detected are represented by *filled circles* and sites that were not occupied by hellbenders are represented by *filled triangles*

Royle, 2005). Regardless, because we did not do repeat surveys in the same year we relaxed the assumption that hellbenders migrate in and out of sites between years; a common assumption of occupancy modeling. We also surveyed two sites only once but both sites produced hellbender captures so we included them in our statistical analyses.

For all captured hellbenders, we determined sex (if possible) (see Petranka, 1998) and measured total length (TL), snout-to-vent length (within 1 cm), and tail width at the base of the tail (within 1 mm). We classified individuals as larvae if they had free gills, juveniles if they lacked free gills but with a TL < 22 cm, and adults if TL > 22 cm (Nickerson & Mays, 1973). To identify recaptured individuals, we injected adult hellbenders with Passive Integrative Transponder (PIT) tags in subcutaneous tissue at the dorsum of the base of the tail. PIT numbers were stored in a PIT tag reader (BioMark Inc, Boise ID, USA). We tagged individuals with TL < 22 cm with Visible Implant Elastomers or VIE (Northwest Marine Technology Inc., Shaw Island WA, USA). We returned all animals to their point of capture after being processed.

Habitat characterization

We recorded stream channel width and selected five 0.25 m^2 quadrats within each transect ($n = 80$ per site). Within each quadrat, we recorded distance to bank, water depth, mid-water column current velocity, and substrate composition. We used a modified Wolman pebble count to quantify substrate size and composition (Wolman, 1954). We measured all lithic particles with diameters >2.0 mm and classified particles >2 m diameter as boulders. We classified non-lithic particles as bedrock, silt, sand, organic matter, or woody debris. We then used these data to calculate medians of particle size and means of stream width, current velocity, depth, and percent non-measurable substrate for each study site.

We assessed water chemistry three times from 2011 to 2013 by measuring DO (mg/l), pH, and conductivity at each site using a YSI Pro Series Multi-Meter (YSI Inc., Yellow Springs, OH, USA). We quantified NO_3^- and NH_4^+ concentrations by taking three to five water samples at each site. Samples were frozen and analyzed within one week of sample collection. Concentrations of NH_3^- and NH_4^+ were determined using an ammonium determination assay (Keeney & Nelson, 1982; Parsons et al., 1984; Mulvaney, 1996) and NO_X^- concentration was determined using manual vanadium (III) reduction (Miranda et al., 2001; Doane & Horwarth, 2003). Although manual vanadium (III) reduction quantifies all variants of NO_X^-, the major contributor to this concentration is NO_3^- and will be referred to as such hereafter. Individual samples were run in triplicate and averaged for each sample and then for each site.

Land-use/land-cover assessment

We quantified upstream land-use and land-cover (LULC) at both the riparian and catchment scales. We quantified Riparian LULC for each site using buffers (100 m) of all upstream tributaries draining into a specific site locality. We clipped the same 2011 National Land Cover Dataset using these buffers and quantified LULC percentages for each site locality. We combined all LULC categories into % forest, % urban, % agriculture, and % grass/shrub prior to statistical analyses. To quantify LULC percentages at the catchment scale, we used a Digital Elevation Model (6.1 m resolution) from the North Carolina Department of Transportation and merged this raster with a National Elevation Dataset (resolution 3 m) from the U.S. Geological Survey (USGS) Geospatial Data Gateway. We used ArcHydro© 10.2 and Spatial Analysis Hydrology toolbox in ArcGIS© 10.2 to delineate upstream watersheds for each site (ESRI, Redlands, CA). We used the 2011 National Land Cover Dataset (resolution 30 m) from the USGS Geospatial Data Gateway and clipped the raster to delineate LULC for each watershed. We then calculated percentages of each LULC category for individual watersheds.

Statistical analyses

We grouped physical and chemical parameters into two classes: habitat (depth, stream velocity, stream width, and substrate) and water-quality (DO, pH, conductivity, NO_3^-). We did not include $NH_4{}^+$ concentration in analyses because NH_4^+ concentration occurred at a detectable level ($\sim$0.1 µg/ml) at only two sites. Using Principal Components Analysis (PCA), we reduced habitat and water-quality parameters into orthogonal variables (PCs). We performed PCA separately on the two parameter classes. We selected PCA because datasets of this nature are often inter-correlated and this analysis produces standardized, orthogonal variables that conform to the assumptions of regression models. Additionally habitat parameters were not significantly correlated with any water-quality parameters ($P > 0.05$). We used a generalized linear model with a binary logistic distribution to determine whether upstream forest cover and habitat and water-quality PCs create a predictive model of hellbender site occupancy. We also attempted to determine predictors of hellbender abundance (CPUE) using a generalized linear model Poisson regression but, likely due to low sample size ($n = 10$ sites with hellbenders present), were not able to detect any informative relationships. We conducted statistical analyses using SPSS 22.0 (SPSS Inc., Chicago, IL. USA) and created graphs using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA).

We chose PC_1 Habitat, PC_3 Habitat, PC_1 Water-quality, and upstream catchment forest cover as covariates for model selection. Riparian forest cover did not predict hellbender occupancy as well as catchment forest cover so we only included catchment forest cover in our analyses. Covariates included in the model (e.g., stream size, substrate composition, nutrient levels, upstream forest cover) are frequently used in models for other sensitive aquatic taxa (Heino et al., 2003; Allan, 2004; Helms et al., 2009; Hopkins, 2009). Using these covariates, we ran all possible model iterations and selected best-fit models based on the lowest AIC values (within 1.0 ΔAIC) and then by Model X^2.

Results

Capture data

We detected hellbenders in 9 of our 21 sites (Fig. 1). We detected larvae at two sites, both of which yielded the largest abundances of hellbenders (>12 captures/150 m). We tagged 73 hellbenders over three field seasons with seven recaptures from two sites. Neither of these recaptures were larval or juvenile individuals. Hellbender body-sizes ranged from 6 to 53 cm and skewed toward larger size classes (Table 1).

Principal components analysis

All habitat and LULC data were normally distributed (Shapiro–Wilk $P > 0.05$). Habitat data demonstrated differences in local habitat between occupied and unoccupied sites (Supplementary Material 2). PCA of habitat data produced four PCs that cumulatively explained 72% of the variation in the habitat data (Table 2). PC_1 Habitat explained 25% of the overall variation and percent fine substrates and woody debris loaded negatively on PC_1 and stream width, velocity, and median substrate size loaded positively. PC_2 Habitat explained 18% of the remaining variation in habitat. Stream depth and percent boulder habitat loaded positively on PC_2 and median substrate size. PC_3 Habitat explained 17.5% of the overall variation. Particle size and percent bedrock loaded positively on PC_3 Habitat while percent organic and fine substrates loaded negatively. PC_4 Habitat explained 13% of the remaining variation in habitat. Stream depth, percent organic substrates, and percent bedrock loaded positively on PC_4 and stream velocity loaded negatively. PCA of water-quality parameters produced two PCs that explained 88% of the total variation (Table 2). Conductivity, NO_3^-, DO, and pH loaded positively on PC_1 water-quality. Conductivity and NO_3^- concentrations loaded positively on PC_2 water-quality and DO and pH loaded negatively.

Generalized linear models

Logistic analysis produced a group of significantly predictive models of hellbender site occupancy. There were no interactions among covariates in all logit models ($P > 0.05$). The three best-fit models were (PC_3 Habitat, Catchment Forest Cover), (PC_3 Habitat), and (PC_3 Habitat, PC_1 Water-Quality) (Table 3). In all models, PC_3 Habitat was responsible for the most variation within the model (Table 4). Locations with hellbenders present had greater particle size and percent bedrock as well as reduced percentages of organic and fine substrates (i.e., higher PC_3 Habitat scores), greater catchment forest cover, and lower NO_3^-, conductivity, and pH (i.e., lower PC_1 Water-Quality scores) (Fig. 2).

Table 1 Number, catch-per-unit-effort (CPUE), and mean (SD) and range of hellbender total lengths (TL) collected from 7 sites in the Watauga River Drainage in 2011 and 2012

Site number	No. captures	Total length (TL)		Recaptures	Larvae/Juveniles	CPUE
		$\bar{X}$ (SD)	Range			
1	33[a]	40.49 (9.82)	6–53	4	5	0.748
2	3[b]	49 (–)	49	0	0	0.126
3	1	53	53	0	0	0.111
4	3	47 (2.83)	45–49	0	0	0.267
5	4	38 (7.35)		0	0	0.344
6	2	45.5 (–)	42–49	0	0	0.386
7	32	37.41 (7.93)	8–46	3	3	0.831
8	1	52 (–)	52	0	0	0.133
9	1	18 (–)	18	0	0	0.118
Total	80	38.68 (9.07)	6–53	7	8	

[a] Three larvae were observed in Site 1 but not captured and are included in the table

[b] The two hellbenders from Site 2 in 2012 were not captured

Table 2 Loading factors and percent variance explained for principal components analysis of all PC groups. Underlined values represent loading factors with absolute values >0.3

	Variable	Component			
		1	2	3	4
Habitat					
	Depth	0.374	0.709	0.299	0.382
	Stream velocity	0.717	−0.019	−0.188	−0.352
	Median substrate	0.489	−0.630	0.295	−0.237
	Stream width	0.799	−0.140	0.064	0.441
	% Wood	−0.447	−0.038	0.392	0.266
	% Bedrock	−0.019	−0.200	0.581	0.458
	% Organic	0.484	0.066	−0.664	0.399
	% Boulder	−0.047	0.801	−0.084	−0.295
	% Fine substrates	−0.516	−0.151	−0.645	0.346
	% Variation explained	24.82	18.11	17.43	12.96
Water-quality					
	NO_3^-	0.761	0.529	–	–
	DO (mg/l)	0.815	−0.479	–	–
	Conductivity	0.711	0.599	–	–
	pH	0.746	−0.588	–	–
	% Variation explained	57.68	30.36	–	–

Discussion

Land-use change often has negative effects on adjacent lotic ecosystems (Allan, 2004). This is most likely because development, agriculture, and logging all require some amount of forest cover removal. As we reduce forest cover in riparian zones and catchments of streams, water and habitat quality may become

Table 3 Model selection for hellbender site occupancy

Model	ΔAICc	w_i	Model X^2	Model P	n
PC_3 Habitat; Catchment Forest	0	0.20	11.74	0.003	21
PC_3 Habitat	0.41	0.15	9.34	0.002	21
PC_3 Habitat; PC_1 Water-quality	1.27	0.15	10.48	0.005	21
PC_3 Habitat; Catchment Forest; PC_1 Water-quality	1.59	0.09	12.16	0.007	21
PC_3 Habitat; Catchment Forest; PC_1 Habitat	1.81	0.09	9.94	0.007	21
PC_3 Habitat; PC_1 Habitat	1.96	0.08	11.78	0.008	21

PC_3 Habitat is included in all best-fit models suggesting that local habitat is the strongest predictor of site occupancy

Table 4 Three best-fit generalized linear model iterations of PC_3 Habitat, PC1 Water-quality, and Catchment Forest Cover on hellbender site occupancy organized by AIC value (smallest to largest)

Model	Covariate	n	Wald X^2	P
PC_3 Habitat, Catchment Forest	Intercept	–	2.13	0.15
	PC_3 Habitat*	21	3.89	0.04
	Catchment Forest	21	2.05	0.15
PC_3 Habitat	Intercept	–	0.55	0.46
	PC_3 Habitat*	21	5.47	0.01
PC_3 Habitat, PC_1 Water-quality	Intercept	–	0.16	0.69
	PC_3 Habitat*	21	4.40	0.03
	PC_1 Water-quality	21	0.81	0.37

PC_3 Habitat is the greatest contributor to all models (marked with asterisks (*)). This suggests that local habitat is a reasonable predictor of hellbender presence/absence

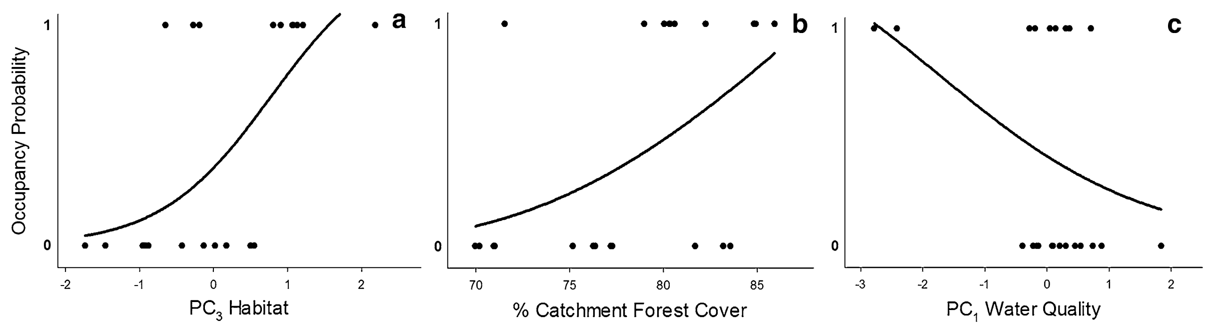

Fig. 2 Response curves of occupancy probability and PC_3 Habitat, percent upstream catchment forest cover, and PC_1 Water-Quality. Occupancy is positively related to PC_3 Habitat and upstream forest cover and negatively related to PC_1 Water-Quality

degraded. Studies of other southern Appalachian streams demonstrated that forest removal causes either immediate or delayed changes in flow discharge (Douglass & Swank, 1975), water temperature (Swift & Messer, 1971), nutrient cycling (Todd et al., 1975), and sediment yield (Swank et al., 2001). Moreover, it has been widely shown that these changes in water and habitat quality often lead to the decreased diversity and richness of stream biota (Harte, 2001; Huston, 2005).

A preponderance of evidence suggests that hellbender declines are dramatic and geographically widespread (Mayasich et al., 2003; Wheeler et al., 2003; Briggler et al., 2007b; Foster et al., 2009; Burgmeier et al., 2011; Graham et al., 2011) leading to the classification of the Ozark hellbender (*Cryptobranchus a. bishopi*; Grobman) as endangered under the U.S. Endangered Species Act (USFWS 2011). Hellbender decline has many plausible sources including prevalence of disease (i.e., *Ranavirus;* Granoff, and *Batrachochytrium dendrobatidis*; Longcore) (Briggler et al., 2007a, 2008; Bodinof et al., 2011; Geng et al., 2011; Souza et al., 2012), introduction of non-native predatory fishes (i.e., Brown and Rainbow Trout) (Gall & Mathis, 2010), and some notable instances of illegal collection (Nickerson & Briggler, 2007). However, our study as well as other recent work suggests that land-use changes may be the primary cause of hellbender declines (Keitzer et al., 2013; Pugh et al., 2013; Quinn et al., 2013).

Our models demonstrate that local physical and chemical parameters and upstream forest cover are strong predictors of hellbender occupancy even in a highly forested catchment (Table 3; Fig. 2). Because the study drainage has good to excellent water-quality, our data suggest that even relatively subtle changes to stream physical and chemical parameters may affect hellbender populations. Given that many other streams throughout this species' range drain catchments that are far more developed than those considered here, it is not surprising that eastern hellbenders have also been recently proposed for protection under the U.S. Endangered Species Act. Moreover, our data provide further evidence that hellbender presence is a reliable indicator of excellent stream habitat and water-quality (Smith, 1907; Hillis & Bellis, 1971; Nickerson & Mays, 1973; Nickerson et al., 2003; Wheeler et al., 2003; Briggler et al., 2007b; Hopkins & DuRant, 2011; Keitzer et al., 2013; Quinn et al., 2013). Because hellbenders generally live much longer than other lotic taxa (30+ years), grow slowly and typically recruit small percentages of larval hatchlings (Nickerson & Mays, 1973), our data suggests that even short-term disturbances or slight alterations to habitat or water-quality may affect population dynamics. Therefore, the hellbender may be a more appropriate indicator species than other taxa in montane lotic systems.

Generalized linear models indicate that PC_3 Habitat was the most informative contributor to all best-fit models (Table 4). Consequently, although water-quality and LULC parameters are adequate predictors of occupancy, local habitat is likely the most important factor influencing hellbender presence/absence. Because stream particle size and percent bedrock loaded positively on PC_3 Habitat and percent organic and fine substrates loaded negatively, this suggests that hellbenders are more likely to be found in sites with larger rocks, more bedrock, and less organic matter and fine sediments. This result is plausible considering hellbenders are typically found in cavities formed under large rocks and in bedrock fractures (see Petranka, 1998). Increased organic and fine substrates may fill interstitial cavities making them less suitable for hellbenders. However, decreased upstream forest cover results in elevated concentrations of fine substrates in stream channels (Kearns et al., 2005; Mancini et al., 2005) so upstream forest cover likely has a significant, possibly indirect, effect on local habitat conditions and, therefore, hellbender site occupancy.

Catchment forest cover and PC_1 water-quality also predicted hellbender site occupancy. No best-fit model included the two covariates together, likely because the strong negative relationship between catchment forest cover and PC_1 water-quality (Pearson $R = 0.50$; $P = 0.01$). This inverse relationship likely decreased the ability of the models to discern the importance of catchment forest cover and PC_1 water-quality. However, such co-variation is likely an inevitable consequence of human development on catchment and stream parameters. Stream reaches with reduced upstream forest cover frequently have higher concentrations of NO_3^- and elevated conductivity compared to more forested reaches (Likens et al., 1970; Webster et al., 2000). Our data demonstrate that LULC is a strong predictor of water-quality even at small spatial scales and when stream water chemistry parameters fall within good to excellent ranges of NO_3^-, DO, conductivity, and pH according to EPA standards (Supplementary Material 3). However, while water-quality and upstream forest cover are informative predictors of hellbender occurrence, it is apparent that local habitat quality is the best predictor of hellbender site occupancy (Table 4).

Analysis of LULC data suggests that hellbenders are acutely sensitive to subtle changes in forest cover. Forested catchments and riparian zones protect water-quality by slowing nutrient export and spiraling rates and attenuating the power of hydrologic events (Allan, 2004; Carpenter et al., 2011; Maloney & Weller, 2011). Few hellbenders were found in catchments with <80% forest cover. Although this level of sensitivity is alarming, it also suggests that buffer zones and selective re-forestation may help mediate the effects of recent ex-urban development and that re-forestation is one of the most promising strategies to restore degraded catchments and adjoining hellbender streams.

Hellbender occurrence is highly variable in the study drainage (Fig. 1). The patchy distribution of hellbenders in our study drainage may also indicate land-use mediated extinction debt is occurring within this drainage (Kuussaari et al., 2009; Jackson & Sax, 2010). The majority of animals that we captured occurred at two sites (Table 1). Other sites produced only one to three captures of large, presumably older animals during repeated sampling events. Because reproduction appears uncommon in many reaches (i.e., larvae were not detected despite substantial search effort targeting larval habitats; Pugh, unpublished data) in many reaches, these sub-populations seem unlikely to persist. However, this could also be due to other factors including pathogens such as *Batrachochytrium dendrobatidis* which is prevalent in this drainage (Williams & Groves, 2014). Further research will be required to confirm these speculations through creating more accurate habitat and land-use models and also by testing these models using data collected from other drainages with patchy distributions of hellbenders.

Although there may have been sites where hellbenders were present and we were not able to detect them, site occupancy was positively related to PC_3 Habitat (Fig. 2). This relationship suggests that we were more likely to find hellbenders in sites that had larger rocks and higher percentages of bedrock substrate; habitats that typically limit the ability of researchers to capture animals during surveys (Nickerson & Krysko, 2003). We also did not search for hellbenders at night or during periods of limited visibility when turbidity increases after large rain events. Future research should incorporate better estimations of detection probability into study design (i.e., more surveys at the same sites) to allow for more accurate and robust occupancy models.

Our data provide the first quantitative link between current landscape (LULC) parameters and local-scale habitat conditions with current hellbender site occupancy. These relationships suggest that land-use changes in Central and Eastern U.S. have led to modification of stream physical and chemical parameters and hellbender habitats. Hellbender declines are alarming because hellbenders are important components of stream communities and likely impact the trophic stability of lotic systems (Smith, 1907; Hillis & Bellis, 1971; Nickerson & Mays, 1973; Humphries & Pauley, 2005). Moreover, because hellbenders are indicator species declines suggest deteriorating water-quality in many headwater systems of the Central and Eastern U.S. Headwaters significantly influence regional water-quality and quantity, thus; deteriorating water-quality may reduce the extent and value of downstream ecosystem services available to human populations (Lowe & Likens, 2005; Wipfli et al., 2007). Therefore, protecting the resources that hellbenders will ultimately provide both health and economic benefits to human populations.

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