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Stream Characteristics Associated with Site Occupancy by the Eastern Hellbender, *Cryptobranchus alleganiensis alleganiensis*, in Southern West Virginia

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Abstract - *Cryptobranchus alleganiensis alleganiensis* (Eastern Hellbender) is an environmentally sensitive species that has experienced range-wide population declines. Diurnal rock-turning surveys were conducted in southern WV during the summer and fall of 2006 to assess the species' population status in this area and to examine the relationship between stream physico-chemical characteristics and site occupancy. Survey results suggest that Eastern Hellbender populations are rare in southern WV, with Eastern Hellbender present at only ≈15% of all sites surveyed and only ≈20% of sites where they have been documented historically. Logistic regression models showed that presence of increased gravel substrate and specific conductivity reduced the probability of site occupancy by Eastern Hellbenders. It is not clear why a higher proportion of gravel substrate negatively affected site occupancy, because gravel should benefit Eastern Hellbender populations by providing larval habitat and habitat for prey species. The effect of specific conductivity may indicate a negative impact of watershed disturbance on populations. This explanation is supported by a principal component analysis of habitat characteristics followed by logistic regression, which demonstrated that sites with habitat characteristics indicative of more degraded sites (e.g., higher specific conductivity) decreased the probability of a site being occupied by Eastern Hellbenders. The results of our study suggest that Eastern Hellbender populations may be severely threatened in southern WV and that site occupancy by Eastern Hellbenders is related to both the physical nature of stream substrate and to water quality characteristics. Furthermore, this study indicates a need for research investigating the potential for human land-use to adversely affect Eastern Hellbenders.

Introduction

Habitat alteration as a result of human activities is often implicated as a cause of global amphibian population declines (Halliday 2005). Unfortunately, habitat requirements of many amphibian species are poorly understood, making it difficult to predict the impact of habitat alteration on the long-term survival of a species (Halliday 2005). *Cryptobranchus alleganiensis* (Daudin) (Hellbender), is an example of an amphibian species that has experienced population declines in most of its range (Burgmeier et al. 2011, Gates et al. 1985, Nickerson and Mays 1973, Nickerson et al. 2002, Pfingsten 1990, Trauth et al. 1992, Wheeler et al. 2003, Williams et al. 1981). Although habitat degradation is believed to be a major reason for these declines (Nickerson and Mays 1973, Wheeler et al. 2003), there have been few studies that address the stream habitat characteristics associated with Eastern Hellbender populations (but see Hillis and Bellis 1971, Humphries and Pauley 2005, Nickerson

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and Mays 1973, Nickerson et al. 2003). Thus, to improve conservation efforts, there is a need for an improved understanding of the habitat characteristics that influence the distribution of Eastern Hellbenders.

C. a. alleganiensis (Daudin 1803)(Eastern Hellbender), is found in streams in the eastern and central United States (Petranka 1998). In general, these animals require cool, fast-flowing streams with a heterogeneous substrate (Nickerson and Mays 1973, Nickerson et al. 2003). Nickerson and Mays (1973) suggested likely optimal conditions for Eastern Hellbenders: water temperatures of 9.8–22.5 °C, pH of 7.6–9.0, and dissolved oxygen concentrations of 8.4–13.6 mg L^{-1}. It also appears that ontogenetic shifts in microhabitat preferences occur (Nickerson et al. 2003). Adults prefer large flat rocks for cover and nesting, which they actively defend, and large rocks may, therefore, be a limiting resource (Nickerson and Mays 1973). In contrast, larvae typically utilize gravel, cobble, and associated interstitial spaces for cover (Nickerson and Mays 1973, Nickerson et al. 2003). These areas also provide habitat for a variety of aquatic invertebrates (Bourassa and Morin 1995, Williams 1978), which make up the bulk of the Hellbender diet (Nickerson and Mays 1973). Therefore, Eastern Hellbender distribution is likely determined by both the water chemistry and substrate characteristics of a stream.

Hellbenders were considered abundant in WV during the early to mid-1900s (Green 1934, Nickerson and Mays 1973), but are currently characterized as very rare or imperiled by the WV Division of Natural Resources (WV Division of Natural Resources 2008). A better understanding of habitat requirements is necessary to identify habitat for protection as well as to locate potential streams for reintroduction efforts. The following study was conducted to gain a better understanding of local habitat characteristics associated with the Eastern Hellbender and examine their population status in southern WV.

Study Area

Surveys were concentrated in southern WV, an area that has not been extensively surveyed in the last 50 years. The survey area encompassed approximately 15,120 km^2 and included a large number of streams that have historically contained Eastern Hellbender populations (Fig. 1). Eastern Hellbenders have been found in the Cranberry River, Williams River, Gauley River, Greenbrier River, East and West Forks of the Greenbrier River, Elk River, Back Fork of the Elk River, Mud River, North Fork of the Cherry River, Guyandotte River, Second Creek, Glade Creek, and Twelvepole Creek in southern WV (Fig. 1). We sampled sites in each of these rivers, as well as in Indian Creek, New River, Second Creek, Bluestone River, Camp Creek, Panther Creek, Dry Fork, Clear Fork, Pond Fork of the Little Coal River, Cherry River, Marsh Fork of the Big Coal River, Anthony Creek, Indian Creek, Mountain Creek, Elkhorn Creek, East River, Dry Fork of the Tug Fork River, Pain Creek, Birch River, Left Fork of the Holly River, Holly River, and Meadow River. Exact sites where populations had been found in previous studies were surveyed if possible. In streams where no population location information was available, sites were subjectively chosen based on their accessibility, our ability to sample (i.e., we only sampled streams shallow enough for us to wade), and if they appeared to contain suitable habitat according to known habitat preferences of Eastern Hellbenders (e.g., heterogeneous stream substrate, cool water temperatures, and swift-flowing water). While this may have biased our results, an examination of the habitat characteristics measured indicates that a wide range in values was sampled (Table 1).

Methods

Surveys were conducted from May through November 2006. Sites were searched by 1 or 2 surveyors wearing snorkeling gear (mask, snorkel, and wetsuit if needed). A log peavey was used to pry up all rocks >250 mm (longest dimension) which were slowly turned to limit disturbance to the substrate. The exposed area was then searched carefully for Eastern Hellbenders while snorkeling. Sites were searched until an individual was encountered or for at least 3 person hours if none were found.

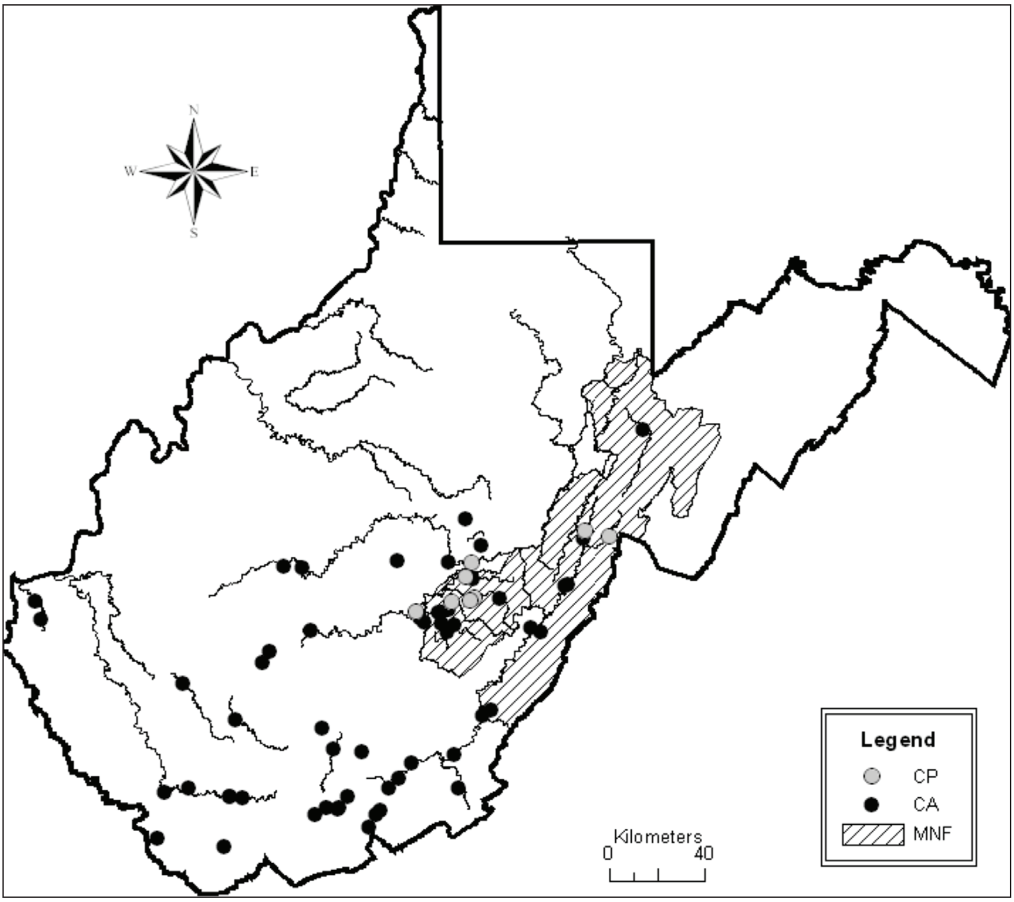

Figure 1. Streams in West Virginia where Eastern Hellbenders have been found in past studies. Gray circles indicate sites surveyed in the current study where Eastern Hellbenders were present (CP sites); black circles indicate sites surveyed but no Eastern Hellbenders were found (CA sites). MNF = Monongahela National Forest.

Physical composition of the stream substrate was characterized by randomly choosing 100 substrate particles along a 100-m transect, following methods similar to the pebble count method described by Wolman (1954). We began our transects from either stream bank at the downstream end of a sampling reach. We stretched a measuring tape across the stream at an angle to the opposite bank in an upstream direction, which ensured that particles were sampled across the entire stream. A researcher walked along the transect and blindly selected by hand a substrate particle every meter. The longest axis of the particle was measured using either measuring tape or vernier calipers, depending on the particle size. The number of particles in a size category was used to determine the proportion of sand (particles less than 2 mm), gravel (particles between 2 mm and 64 mm), cobble (particles between 64 mm and 256 mm), and boulder (particles greater than 256 mm) at each site (Bunte and Abt 2001). Stream substrate heterogeneity was calculated using the Shannon-Wiener index of diversity (H'), defined as $H' = -\sum p_i (\ln p_i)$, where p_i is the proportion of each substrate category. Water variables were measured with a Hydrolab Quanta (Hydrolab Corporation, Austin, TX) and included water temperature (°C), pH, percent dissolved oxygen, specific conductivity (µS cm^{-1}), and turbidity (NTU). Relative abundance of crayfish (number of crayfish observed/person hour) was also recorded because crayfish make up a significant portion of the Eastern Hellbender diet (Alexander 1927, Green 1933, Netting 1929, Nickerson and Mays, 1973 Reese 1903).

Shapiro-Wilk's tests for normality and visual examinations of binned residuals of logistic regressions were performed to check for normality and constant variance of habitat characteristics. Data that failed to meet normality and constant variance assumptions were either natural log transformed (relative abundance of crayfish, specific conductivity, percent dissolved oxygen), square-root transformed (turbidity), or arcsine square-root transformed (proportion of sand, gravel, cobble, and boulder substrate). Data were then standardized for further analyses such that they had a mean of zero and a standard deviation of one.

Table 1. Range of values observed for stream habitat parameters. The numbers in parentheses are for sites where Eastern Hellbenders were present during surveys.

Parameter	Minimum	Maximum	Mean	SD
Crayfish (no./person-hr)	0 (0)	43.67 (21.07)	6.43 (6.93)	7.35 (7.31)
Water temperature (°C)	7.39 (14.82)	29.59 (20.06)	20.23 (17.71)	5.52 (1.85)
Specific conductivity (µs/cm)	29 (29)	1043 (53)	200 (36)	210 (8)
Dissolved oxygen (mg/L)	6.1 (7.33)	11.15 (9.61)	8.68 (8.44)	1.06 (0.82)
pH	5.79 (5.83)	8.42 (7.01)	7.21 (6.42)	0.69 (0.42)
Turbidity (NTU)	3.03 (3.03)	73.27 (29.33)	29.66 (17.36)	15.5 (9.38)
% sand	0 (1)	22 (15)	4.2 (6.25)	5.57 (5.99)
% gravel	10 (10)	53 (24)	30.79 (16.25)	10.64 (5.04)
% cobble	11 (16)	49 (44)	32.3 (35.38)	10.12 (10.54)
% boulder	13 (28)	59 (59)	31.48 (42.13)	10.45 (9.61)
Substrate heterogeneity (H')	0.99 (0.99)	1.36 (1.36)	1.14 (1.14)	0.09 (0.09)

We examined the effects of local habitat characteristics on site occupancy by Eastern Hellbenders using logistic regression with a binomial error distribution. We recognize that not accounting for detection probability may bias our results, but our study design did not include multiple revisits to a large number of sites, which would have been necessary to estimate detection probability (MacKenzie et al. 2002). When using a binary response variable (i.e., presence/absence) it is recommended to include $\leq m/10$ explanatory variables, where m is the least frequent category of the binary response variable. We observed Eastern Hellbenders at a small number of sites ($m/10 = 0.8$) which limited the number of covariates that could be included in our models to one. As a result, we could not model potential additive effects or include interactions among habitat variables in our analysis. We therefore conducted a principal component analysis (PCA) to reduce habitat characteristics into uncorrelated variables. All of the transformed and standardized habitat characteristics were included in the PCA, which was based on the correlation matrix. Frontier's Broken Stick Criterion was used to determine which principal components should be kept for further analyses (Legendre and Legendre 1998). The effects of principal components were then analyzed using logistic regression with a binomial error distribution.

Akaike's information criterion (AIC) corrected for small sample size (AIC_C) was used to rank models (Burnham and Anderson 2002). We considered models with AIC_C scores less than seven units from the model with the lowest AIC_C score to be potential models for explaining Eastern Hellbender site occupancy. Analyses were performed using R version 2.9.2 (R Core Development Team 2009).

Results

We observed Eastern Hellbenders at 8 of 58 sites, which included 41 sites at which they had been found during previous surveys. Stream habitat characteristics ranged widely at these sites, but streams were generally cool and well oxygenated with a heterogeneous substrate (Table 1). The first two principal components were kept for further analysis based on Frontier's Broken Stick Criterion. Variable loadings on the first axis suggested that it described a gradient of disturbance, and habitat characteristics associated with more disturbed sites were negatively loaded on this axis (Table 2). The second axis described differences in substrate, particularly the proportion of sand substrate and substrate diversity, which were positively loaded on this axis, versus the proportion of boulder habitat (Table 2).

The best set of models based on AIC_C values included the proportion of gravel substrate, specific conductivity, and the first principal component axis (PC 1) as covariates (Table 3). Estimates of these coefficients suggest that increases in the proportion of gravel substrate and specific conductivity at a site reduced the probability of a site being occupied, while increases in PC 1 had a positive effect on the probability of a site being occupied (Fig. 2, Table 3).

Discussion

The physiology and behavior of Eastern Hellbenders suggest that abiotic factors play an important role in shaping their abundance and distribution. This conclusion has led many researchers to identify habitat degradation as a potentially important factor in recent declines in Eastern Hellbender populations (Nickerson and Mays 1973, Wheeler et al. 2003). Therefore, it is critical to understand the habitat characteristics required by Eastern Hellbenders to identify appropriate areas for conservation and potential reintroduction. We surveyed streams representing a gradient of stream habitat characteristics to assess influence of habitat characteristics on the presence of Eastern Hellbenders. Our results suggest that abiotic factors are important in determining the presence of these

Table 2. Loadings of habitat characteristics along the first two principal component axes.

	Principal components	
Habitat characteristic	PC 1	PC 2
Crayfish	0.15	0.33
Water temperature	-0.29	-0.29
Specific conductivity	-0.44	0.12
pH	-0.48	<0.10
Dissolved oxygen	-0.31	<0.10
Turbidity	-0.37	-0.27
Sand	-0.10	0.52
Gravel	-0.33	-0.33
Cobble	0.26	0.26
Boulder	0.18	0.18
H'	-0.14	-0.14

Table 3. Results of model selection based on AIC_c values. The number of parameters (K), change in AIC_c value (Δ AIC_c), model weight (ω_i), cumulative model weight ($\Sigma\omega_i$), log-likelihood (LL), and parameter estimate (β) are shown.

Model	K	AICc	Δ AICc	ωi	Σωi	LL	β
Gravel^A	2	26.10	0.00	0.68	0.68	-10.94	-3.13
Specific conductivity^A	2	27.86	1.76	0.28	0.97	-11.82	-5.55
PC 1^A	2	32.32	6.22	0.03	1.00	-14.05	1.26
pH	2	37.45	11.35	0.00	1.00	-16.62	-1.68
Boulder	2	41.23	15.13	0.00	1.00	-18.51	1.26
Turbidity	2	43.52	17.43	0.00	1.00	-19.65	-1.10
Dissolved oxygen	2	45.65	19.55	0.00	1.00	-20.71	-0.90
Sand	2	48.47	22.37	0.00	1.00	-22.12	0.56
Null	1	48.61	22.51	0.00	1.00	-23.27	-
Water temp.	2	48.81	22.71	0.00	1.00	-22.29	-0.54
Cobble	2	49.90	23.80	0.00	1.00	-22.84	0.37
Crayfish	2	50.54	24.44	0.00	1.00	-23.16	18.00
PC 2	2	50.70	24.60	0.00	1.00	-23.24	0.06
H'	2	50.76	24.66	0.00	1.00	-23.27	0.01

^{A}Models included in the best set.

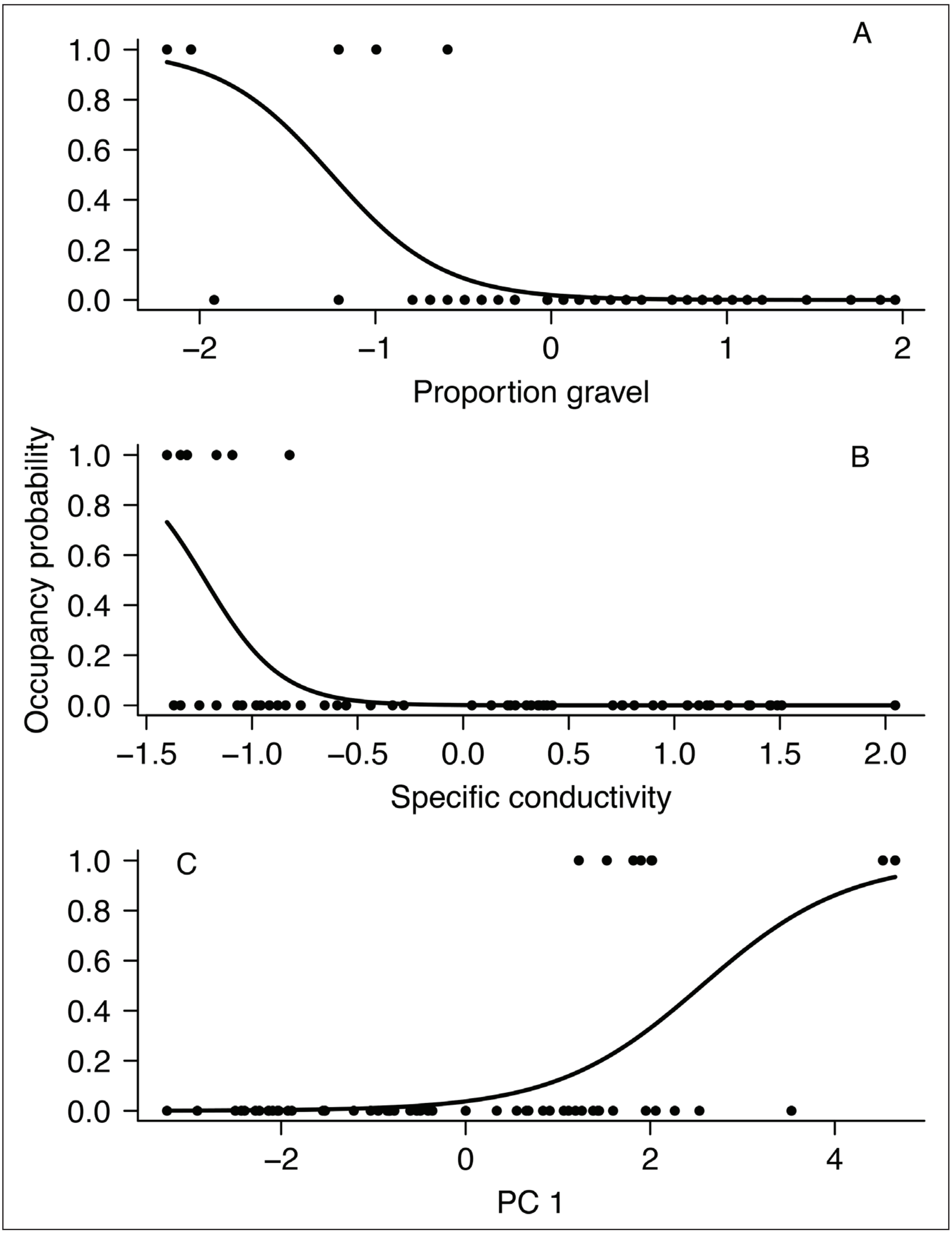

Figure 2. Effects of gravel (A), specific conductivity (B), and the first principal component (C) on site occupancy by Eastern Hellbenders. Circles represent observed site occupancy by Eastern Hellbenders. Gravel was originally measured as the relative proportion of gravel substrate at a site, but was arcsine square-root transformed and standardized for the analysis. Specific conductivity (µS cm^{-1}) was natural log transformed and standardized for the analysis. Principal component one (PC 1) is unitless and was not standardized for the analysis.

animals, and that Eastern Hellbender distribution may be influenced by landscape disturbances that alter local habitat characteristics.

Based on the fact that we found them at only <15% of the survey sites, Eastern Hellbenders appear to be rare in southern WV. This apparent rarity is troubling because Hellbender populations were once common in WV (Green 1934), yet we failed to detect them at ≈80% of the sites in rivers where they have been found in the past. This finding may indicate population declines in southern WV, adding to the considerable evidence that Eastern Hellbender populations are declining throughout the species' range (Burgmeier et al. 2011, Gates et al. 1985, Nickerson and Mays 1973, Pfingsten 1990, Trauth et al. 1992, Wheeler et al. 2003, Williams et al. 1981). Our results may also reflect the difficulty of detecting a rare and secretive species, thus highlighting the need for further population surveys in this area to firmly establish the population status of Eastern Hellbenders in southern WV.

It is interesting to note that 88% of the sites where we found Eastern Hellbenders are located within or near the Monongahela National Forest (MNF), suggesting that this area provides some protection for Eastern Hellbender populations. While the MNF may provide protection from a variety of stressors, the protection it provides from landscape disturbance is likely a key factor for conserving Eastern Hellbender populations. Land-use practices can drastically alter abiotic stream characteristics at both local and watershed scales, and this degraded habitat can negatively impact aquatic biota (reviewed by Allan 2004). The negative effect of increasing specific conductivity on site occupancy by Eastern Hellbenders provides some support for this hypothesis. While specific conductivity can be influenced by watershed geology, Dow and Zampella (2000) found that specific conductivities in the range of 70–140 μS cm^{-1} were associated with streams draining watersheds characterized by detrimental land uses (e.g., agriculture, logging, etc.). Urbanization, agricultural practices, mining, and logging can increase the amount of nitrate, ammonium, phosphorus, calcium, sulfate, and magnesium present in streams, which results in a higher conductivity, a condition that can negatively impact aquatic biota and ecosystem processes (Dow and Zampella 2000, Lenat and Crawford 1994, Paul and Meyer 2001, Pond et al. 2008, Sponseller and Benfield 2001). Sites where Eastern Hellbenders were found had a mean specific conductivity of 36 μS cm^{-1} and a maximum value of 53 μS cm^{-1}, values that are indicative of relatively undisturbed watersheds (Dow and Zampella 2000). Conversely, sites where Eastern Hellbenders were absent had a much higher mean specific conductivity (230 μS cm^{-1}), possibly due to a high level of large-scale disturbance in those watersheds. These results suggest that studies examining the impacts of large-scale disturbance on Eastern Hellbender populations are needed.

The negative impact of abundant gravel substrates on Eastern Hellbender site occupancy is difficult to interpret. Gravel substrates are generally associated with high macroinvertebrate diversity and abundance (Allan 1997, Rabeni and Minshall 1977, Reice 1980, Williams 1978), which should benefit Eastern Hellbenders by providing a plentiful and diverse prey base. Additionally, gravel substrates provide habitat for larval Eastern Hellbenders (Nickerson et al. 2003). It was surprising, therefore, that increases in the proportion of gravel substrate decreased the probability of site occupancy by Eastern Hellbenders. It is possible that smaller size particles included in the gravel size class (2–64 mm) may limit the animals' access to large rocks and boulders. Hellbenders, particularly adults, utilize boulders as nesting habitat and cover objects during the day, and their availability may be a limiting resource (Nickerson and May 1973). Thus, limited availability of large rocks and boulders could have long-term negative consequences for Hellbender populations. However, more research is needed to determine if this is the case, and we recommend caution in interpreting the significance of this result.

The positive effect of the first principal component suggests that factors positively loaded on this access benefited Eastern Hellbender populations. This result indicates that sites with a high abundance of crayfish and a large proportion of cobble and boulder substrate support Eastern Hellbender populations. In contrast, sites with higher water temperature, specific conductivity, pH, dissolved oxygen, turbidity, proportion of sand and gravel substrate, and substrate diversity are less likely to support Hellbender populations. The majority of these habitat characteristics make biological sense because these animals prefer cool streams, have a diet consisting largely of crayfish, and require large rocks and cobble substrates (Nickerson and Mays 1973, Nickerson et al. 2003). Additionally, many of the characteristics negatively loaded on the first principal component (e.g., higher stream temperature, turbidity, and specific conductivities) are indicative of disturbed watersheds (Allan 2004, Dow and Zampella 2000), suggesting that degraded habitats may not support large Hellbender populations. However, we do not have data regarding historic habitat characteristics for many of the streams we sampled, so it is possible that these sites do not represent degraded habitats, but rather have always been poor sites for Eastern Hellbenders. Regardless, it appears that any site with these habitat characteristics is unlikely to support Hellbenders.

We stress that caution should be used in interpreting and extrapolating our results for at least two reasons. The first is the small number of sites at which Hellbenders were present. This limited the number of covariates and interactions that could be included in models, the inclusion of which may have altered parameter estimates and the inferences about the effects of the habitat characteristics being tested. The second is that our study design failed to account for imperfect detection. This is potentially problematic for a rare and secretive species such as the Hellbender, which may have been present at a site but was simply missed during surveys. If this were the case, we may have inaccurately estimated the influence of habitat characteristics on Hellbender site occupancy (MacKenzie et al. 2002). We recommend that future population surveys should obtain estimates of detection probability by repeatedly sampling at least a portion of the survey sites as recommended by Mazerolle et al. (2007) for amphibian and reptile species.

Range-wide declines have been observed in Hellbender populations in recent years (Wheeler et al. 2003). It appears that this decline is occurring in southern WV because we rarely encountered Hellbenders during our surveys. While there are undoubtedly numerous factors contributing to the declines, the environmentally sensitive nature of Hellbenders suggests that habitat degradation has played an important role (Nickerson and Mays 1973, Wheeler et al. 2003). The results of our study provide support for this claim, and demonstrate that both water and stream substrate habitat characteristics are important in determining the distribution of Hellbender populations. Furthermore, our results suggest that changes in land use may have altered local habitat conditions and negatively impacted Hellbender populations. Research is needed to investigate the potential effects of human land-use on Hellbenders to improve conservation planning for this unique species.

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