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Source: Herpetologica, 76(4) : 355-365

Published By: The Herpetologists' League

URL: https://doi.org/10.1655/0018-0831-76.4.355

HERPETOLOGICA

VOL. 76 DECEMBER 2020 NO. 4

Herpetologica, 76(4), 2020, 355–365

The Influence of Multiscale Habitat Variables and Population Density on Artificial Shelter Use by Hellbenders (*Cryptobranchus alleganiensis*)

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Abstract: Recently, artificial shelters have been proposed as a novel tool to monitor Hellbenders (*Cryptobranchus alleganiensis*) and other cryptobranchid salamanders. Factors that influence artificial shelter use by Hellbenders have not been identified, but are important for maximizing the utility of these shelters as monitoring tools. To identify these factors, in 2013–2018 we deployed 438 artificial shelters across 10 stream reaches inhabited by Hellbenders, within three rivers in the upper Tennessee River Basin. We hypothesized that occupancy and nesting would depend on shelter placement, and would be greatest in reaches with relatively high densities of adult/subadult Hellbenders (i.e., >1.5 individuals per 100 m^2). We placed shelters in locations representing a range of instream conditions, but avoided microhabitats that were not suitable for Hellbenders. We monitored shelter occupancy by Hellbenders every 2–8 wk, and surveyed shelters for nests every 2–5 d during their breeding season. We quantified densities of adult/subadult Hellbenders and 10 habitat variables across multiple spatial scales. Hellbenders occupied 46% of artificial shelters, and nested in 17% of artificial shelters that were in place for at least one breeding season. Hellbenders were most likely to occupy and nest in shelters placed in portions of those reaches that were ≥50 cm deep with high densities of adult/subadult individuals. Among the variables we considered, population density was the most important factor influencing shelter occupancy by Hellbenders. Shelter nesting was most influenced by water depth, but also by population density and time since shelter installation. Both occupancy and nesting in shelters increased for 2–3 yr following shelter deployment. Our results provide evidence that artificial shelters constitute efficient tools in some streams for monitoring the occurrence and reproduction of Hellbenders.

Key words: Amphibian conservation; Aquatic salamanders; Cryptobranchidae; Nest box; Noninvasive sampling; Reproduction; Resource use

Because amphibians are declining globally and are often difficult to detect using existing survey methods, the development of effective monitoring tools and protocols is critical for their conservation. Stream-associated species exemplify this need, because many are declining at an alarming rate (e.g., Mathews and Morgan 1982; Ryan 1998; Ashton et al. 2006; Surasinghe and Baldwin 2015) and some can be notoriously difficult to detect (e.g., Chattin et al. 2007; Browne et al. 2011; Pierson et al. 2016). Artificial shelters have promise as a tool for improving the monitoring of certain stream-associated amphibians because they mimic features of natural instream habitat that are otherwise difficult to access (Briggler and Ackerson 2012; Button et al. 2020; Jachowski et al. 2020). Given that artificial shelters might improve monitoring capabilities for multiple imperiled stream-associated amphibians, their development and use warrants assessment.

Effective monitoring of Hellbenders (*Cryptobranchus alleganiensis*) might be possible using artificial shelters but has not often been demonstrated in practice. Hellbenders are large (up to 74 cm total length), fully aquatic salamanders found across the Appalachian Mountains and portions of the lower Midwest, primarily in cool, fast-moving, well-oxygenated streams with abundant cover rocks and crevices (Alexander 1927; Nickerson and Mays 1973; Beffa 1976; Humphries 1999; Keitzer et al. 2013). Within suitable streams, Hellbenders typically reside beneath large boulders (Keitzer 2007; Da Silva Neto et al. 2019), and often utilize deep portions of the streams they inhabit, especially during hot summer weather (Green 1934). Hellbenders are declining rapidly across much of their range, particularly in impaired watersheds with degraded upstream forest cover (Williams et al. 1981; Briggler et al. 2007; Jachowski and Hopkins 2018). Surrounding land use change is most frequently linked to declines of Hellbenders (Pugh et al. 2016), particularly when it involves the alteration of upstream riparian forests and increased sediment loads (Briggler et al. 2007; Jachowski and Hopkins 2018). The causal mechanisms underlying population declines of Hellbenders are poorly understood, however, partly because adult Hellbenders spend most of their lives beneath large boulders and are, therefore, difficult to study (Topping and Peterson 1985). Researchers have usually studied Hellbenders using rock-lifting surveys, which involve dislodging and overturning boulders in streams (e.g., Jachowski et al. 2020). While effective for detecting Hellbenders, rock-lifting surveys are potentially dangerous for both animal and surveyor, can damage critical instream habitat (Browne et al. 2011), and are ill-advised between August and April, when nests of Hellbenders could be destroyed. Therefore, less invasive alternatives to rock-lifting surveys are needed in order to monitor populations of Hellbenders without exerting undue survey-related pressures on them.

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TABLE 1.—Median, minimum, and maximum daily discharges (in m^3/s) over the period of shelter deployment for each river containing artificial shelter arrays for Hellbenders (*Cryptobranchus alleganiensis*) in the Upper Tennessee River drainage. Values for predictor variables that varied with stream discharge were calculated when the discharge of each river was at its approximate annual median. All River 1 and River 2 study reaches were located upstream of the nearest US Geological Survey (USGS) gauge. On River 1, two study reaches were located 8.72–134 channel km upstream of the nearest USGS gauge, and three were located 0.05–17.72 km downstream of the gauge.

River	Period of shelter deployment	Discharge (m^3/s)		Channel km between USGS gauge and study reaches (range)
		Median	Range (min.–max.)	
1	June 2018–present	2.95	1.64–11.78	23.28–40.77
2	June 2014–present	3.26	0.85–121.20	15.33–22.75
3	May 2013–present	2.38	0.65–51.54	0.05–17.72

The advent of artificial shelters has presented a potential noninvasive alternative to rock-lifting surveys for studying Hellbenders (Briggler and Ackerson 2012). Although less destructive than rock-lifting surveys, the utility of artificial shelters for monitoring Hellbenders has yielded mixed results in practice (cf. Messerman 2014; Button 2019). Whereas improvements have been made to increase the stability and availability of artificial shelters to Hellbenders (Button et al. 2020), it remains unclear whether characteristics of shelter placement within streams influence their use. Because Hellbenders exhibit high site fidelity (Bodinof et al. 2012), spend much of their time beneath their preferred cover rocks (Topping and Peterson 1985; Peterson 1987), and have small home ranges (Blais 1996), it might take at least 2 yr for Hellbenders to discover and use artificial shelters (Jachowski et al. 2020) depending on stream. Longer-term monitoring of shelters is needed to determine the time at which occupancy can be expected to plateau across a range of population densities. Therefore, we sought to determine how time since shelter installation and several multiscale instream variables influenced occupancy and nesting in artificial shelters by Hellbenders. Our study is the first to quantitatively evaluate patterns of artificial shelter use by Hellbenders over several (5+) years, and provides guidance for determining where to place artificial shelters to maximize their utility.

MATERIALS AND METHODS

Study Reaches

We deployed 10 artificial shelter arrays across three rivers in the upper Tennessee River Drainage in southwest Virginia. Hereinafter, we refer to the extent of stream containing an artificial shelter array (range = 206–376 channel m) as a "reach." Because of the conservation status of the focal species, we do not specify precise locations and refer to our study rivers as Rivers 1, 2, and 3. All three rivers were of fourth-order magnitude (Strahler 1952) at our study reaches, although these reaches varied widely in their upstream catchment size (range = 131–309 km^2). When river discharges were at their annual medians (Table 1), our reaches in Rivers 1 and 3 had wetted widths of 14–20 m, while reaches in River 2 had wetted widths of 6–18 m. Our study reaches varied considerably in their level of impairment, as measured by percentage of upstream forest cover in their catchment-wide riparian area (CWR; range = 54–70%; Jachowski and Hopkins 2018). We used the National Landcover Dataset data (USGS 2019) to calculate the forested percentage of landcover within 50 m of all stream reaches upstream of a given study reach. The amount and arrangement of habitat suitable for Hellbenders (i.e., large boulders with appropriate crevices) also varied widely among our study reaches (Table 2), allowing us to evaluate the relative influence of reach- versus microhabitat-scale habitat variables on shelter use. Within each river, we spaced consecutive study reaches an average of 5.5 channel km apart from each other (range = 1.5–14.3 channel km).

Demographics of Hellbenders

Because population density can influence shelter occupancy by Hellbenders (Jachowski et al. 2020), we deployed

TABLE 2.—Mean values and ranges for all variables used in our shelter occupancy and nesting analyses for Hellbenders (*Cryptobranchus alleganiensis*) in the Upper Tennessee River drainage. Variables of binary nature (present/absent) were coded as 0 or 1; therefore, their mean values represent the percentage of shelters where the variables were present.

Variable	Mean, or probability of presence	Range	Variable type	Scale
Adult/subadult density of Hellbenders (individuals/1680 m^2)	23.05	6–51	Continuous	Reach
Habitat surplus (continuous density)	0.05	−0.28 to 0.45	Continuous	Reach
Reach-scale density of large suitable boulders (number per 10 transects across stream width)	6.38	3–14	Count	Reach
Upstream catchment size (km^2)	197.24	131.31–309.00	Continuous	Reach
Upstream catchment-wide riparian area forest cover (%)	63.00	54–70	Continuous	Reach
Channel transition status	0.47	0 or 1	Binary	Core habitat
Pool-riffle-run transition status	0.15	0 or 1	Binary	Core habitat
Sand/gravel bar transition status	0.36	0 or 1	Binary	Core habitat
Bank-to-bank current velocity (m/s)	0.13	0–0.66	Continuous	Microhabitat
Distance to bank (m)	3.70	0.10–9.30	Continuous	Microhabitat
Downstream current velocity (m/s)	0.28	−0.13 to 1.10	Continuous	Microhabitat
Tunnel angle (degrees)	24.65	0.00–105.00	Continuous	Microhabitat
Canopy cover (%)	70.00	0–100	Continuous	Microhabitat
Vertical distance to canopy (m)	3.01	0.19–16.05	Continuous	Microhabitat
Water depth at tunnel (cm)	44.21	19.00–103.00	Continuous	Microhabitat
Crevice-bearing boulders within 1 m	3.38	0–11	Count	Microhabitat
Total breeding seasons since installation (nesting)	2.56	1–6	Count	Temporal
Average months since installation (occupancy)	10.06	1–31	Count	Temporal

artificial shelter arrays in reaches that varied considerably in their density of Hellbenders and demographic structure (Appendix S-1 in Supplemental Material, available online). Most study reaches (4 of 5) in River 3 contained moderate to high adult/subadult densities of Hellbenders (0.65–3.04 individuals/100 m^2) and exhibited a relatively stable population age structure and successful recruitment (Jachowski and Hopkins 2018). In contrast, the most downstream study reach in River 3, and all five study reaches on Rivers 1 and 2, featured low to moderate (0.36–0.64 individuals/100 m^2), or unknown adult/subadult densities of Hellbenders (Jachowski and Hopkins 2018; Appendix S-I, Supplemental Material). Low-density populations of Hellbenders within our study reaches were comprised mostly of old adults, indicating a likely lack of recruitment by juveniles and/or successful reproduction (Jachowski and Hopkins 2018).

Artificial Shelters

We constructed artificial shelters using the designs of Briggler and Ackerson (2012) and Button (2020). Briefly, shelters consisted of a hollow, boot-shaped metal frame encased in 2–4 cm of concrete on all sides. From April–July of 2013–2018, we deployed approximately 30 shelters within each study reach (n = 300 shelters in place at once), and replaced damaged or dislodged shelters as necessary. In total, 438 unique shelters were deployed over the course of the study. Typically, we deployed replacement shelters in different locations and orientations from the shelters that they replaced. We embedded all shelters firmly into the stream substrate during installation, in microhabitats that represented a range of potentially suitable conditions (Table 2). We did not place shelters in microhabitats known to be unsuitable for Hellbenders, however, such as shallow side-pools, leaf packs, and locations with no natural cover objects within 10–20 m. We also avoided orienting shelter tunnels so that they faced upstream, as doing so would have caused the shelters to quickly fill with sediment or become dislodged. We deposited a thin layer of sand and gravel into shelter chambers and tunnels to mimic natural crevices, and spaced adjacent shelters an average of 10 channel m apart from each other (range ≈ 4–20 m) within each reach.

Data Collection

We monitored shelter occupancy for the duration of time that each shelter was in place, and recorded nesting in shelters during each breeding season for Hellbenders. We conducted occupancy surveys every 2–8 wk during the nonbreeding season, except when unfavorable conditions (e.g., floods or ice) made surveys infeasible. To determine if shelters were occupied, we removed the lid while blocking the tunnel entrance, and manually probed the interior. We surveyed shelters for nests every 2–5 d during the breeding season (15 August–20 September), and simultaneously recorded shelter occupancy when doing so.

Given the low vagility of Hellbenders (Topping and Peterson 1985; Blais 1996), we collected habitat data across three spatial scales (Table 2): reach, core habitat (i.e., within ≤5 m of each shelter), and microhabitat (i.e., at or within 1 m of each shelter). Stream reaches are perhaps the largest scale in which Hellbenders select resources, whereas the area encompassed by a 5-m radius around a shelter is similar to the area of core habitat use within home ranges of Hellbenders (Hillis and Bellis 1971; Bodinof et al. 2012). Within their area of core habitat use, Hellbenders occupying artificial shelters likely use microhabitats adjacent to (i.e., at or within 1 m of) those shelters most often, as Hellbenders exhibit high shelter and cover object fidelity (Blais 1996; Bodinof et al. 2012; W.A. Hopkins, personal observations). We also assessed the influence of reach-scale density of Hellbenders and average time since shelter installation across all surveys or breeding seasons on both shelter occupancy and nesting, because the influence of these factors on shelter use for reproduction and on occupancy beyond a 2-yr period is unknown (cf. Jachowski et al. 2020).

Reach scale.—We assessed the influence of three reach-scale habitat variables on shelter occupancy and nesting: upstream catchment size, percentage of forest cover in the upstream CWR area, and density of large boulders with crevices suitable for Hellbenders. Upstream catchment size is known to influence community structure, whereas upstream forest cover is an important mediator of stream impairment and habitat quality (Hooke 2000; Collins et al. 2009; Jachowski and Hopkins 2018). We calculated both upstream catchment size and percentage of upstream CWR forest cover in ArcMap v10.1 (ESRI, Redlands, CA), using the 2011 National Land Cover and National Hydrography Datasets (USGS 2019; most recent versions of data available at time of analysis).

We quantified habitat for Hellbenders at each of our study reaches based on the reach-scale density of boulders that bore crevices suitable for Hellbenders. We counted only boulders that were >40 cm long on their primary axis, because 95% (1002/1056) of captures of Hellbenders detected during rock-lifting surveys in our study system came from beneath boulders this size or larger (Wolman 1954; W.A. Hopkins, personal observation). We considered large boulders suitable for Hellbenders if they met all of the following four criteria: the boulder did not move when nudged, an observer could slide their hand into a crevice under the boulder up to at least their second knuckle (i.e., it was not fully embedded and had a crevice), the crevice was not packed with debris (i.e., sticks and leaves, which are indicative of boulders that are perched high and collect debris), and the crevice lacked apparent connections to other crevices (i.e., it was not possible for an observer to touch their hands together when reaching under separate crevices). To estimate the density of boulders that were both large and suitable at each study reach, we walked 10 evenly spaced transects across a representative 1680-m^2 portion of the reach (Jachowski et al. 2020) and measured every boulder that intersected these transects.

Core habitat scale.—The area enclosed within a 5-m radius around each shelter (78.54 m^2) is similar to the size of the core area within typical home ranges of Hellbenders (Blais 1996; Burgmeier et al. 2011; Bodinof et al. 2012). Therefore, we evaluated the potential influence of three variables within 5 m of each shelter on shelter occupancy and nesting: the presence or absence of a steeply cut channel (>10% incline on both sides), sand/gravel bar (>1 m^2), and pool-riffle-run transition. We hypothesized that these three variables would be related to shelter use by Hellbenders because of their potential influence on the diversity of resources available within an individual's home range. We assessed the presence/absence of all three core habitat-scale

variables through visual estimation, and defined pools, riffles, and runs using the same criteria as Dey (2014). We used a tape measure to determine whether a variable was present within 5 m of a shelter.

Microhabitat scale.—Given that microhabitat features are often important drivers of resource use in nonvagile species (Welsh and Ollivier 1998), we assessed the influence of several microhabitat-scale variables (i.e., at or within 1 m of the shelter) on both occupancy and nesting. We measured the angle formed between the direction of each shelter's tunnel and the direction of stream current ("tunnel angle"); current velocity at the tunnel parallel to, and perpendicular with, the current ("downstream current velocity" and "bank-to-bank current velocity," respectively [m/s]); water depth at the tunnel entrance (cm); shelter distance to the bank (m); percentage of canopy cover above the shelter; vertical distance to canopy (m, where applicable); and the number of crevice-bearing boulders within 1 m of the shelter. To determine tunnel angle, we attached a fishing bobber to the end of a 50-cm-long string, held the opposing end of the string at the water's surface above the base of the tunnel, and measured the angle formed between the string and the tunnel using a protractor. We used a 2D FlowTracker2 Handheld-ADV flow meter (Xylem Inc.) placed at $6/10^{ths}$ the depth of the stream to assess downstream and bank-to-bank current velocity, and measured water depth at the tunnel entrance with a meter stick. Using a tape measure, we calculated distance to bank, then combined this measurement where applicable with a clinometer-based angle to above-shelter canopy taken from the bank to determine vertical distance to canopy. To estimate percentage of canopy cover, we photographed the canopy above each shelter using a fisheye lens (GoPro Inc.), digitally overlaid a densiometer-style grid of 96 dots onto each photo, and multiplied the number of dots that intersected canopy by 1.04 (Lemmon 1956). We considered boulders (b-axis $\geq$ 256 mm; Wolman 1954) within 1 m of shelters to be crevice-bearing using the same criteria used for determining reach-scale boulders. We measured all discharge-dependent variables once, when discharge at the nearest US Geological Survey stream gauge (Table 1; USGS 2019) was at its approximate annual median.

Time since installation and density of Hellbenders.—In addition to being influenced by multiscale habitat variables, we predicted that shelter occupancy and nesting would increase over several years following shelter installation, and increase concomitant with adult/subadult density of Hellbenders. We used average months since shelter installation across all surveys to model shelter occupancy, and used number of breeding seasons since shelter installation to model shelter nesting. To evaluate the influence of density of Hellbenders on shelter use, we used existing density estimates from five of our six multiyear reaches (Hopkins and Jachowski 2018), and estimated density at the sixth using a single-season Huggins closed-capture model (Huggins 1989) in 2018 (Appendix S-I, Supplemental Material). In this instance, we considered detection across our two surveys of the reach constant given the short timespan between these surveys, and variable between individuals. Reach-scale densities of large boulders bearing suitable crevices explained 80% of the variation in densities of Hellbenders across our study reaches (W.A. Hopkins, personal observation).

Shelter design.—Button et al. (2020) reported that artificial shelters should be constructed with thick walls, heavy frames, and inset lids to improve stability during high stream discharge events. Therefore, we assessed whether Hellbenders occupied and nested in shelters built using a heavy, sturdy design (n = 220) as often as shelters constructed using the original, more lightweight design (n = 218; Briggler and Ackerson 2012). In doing so, we sought to verify that no tradeoff existed between shelter stability and shelter use.

Data Processing and Analyses

Response units.—We used average occupancy and nesting at individual artificial shelters as our response variables in all analyses. To estimate average occupancy by Hellbenders at each artificial shelter, we divided the number of surveys in which we found each shelter occupied (n = 0–64) by our total number of surveys of the shelter (n = 1–76). To calculate average shelter nesting, we divided the number of breeding seasons in which Hellbenders nested in each shelter (n = 0–4) by the total number of breeding seasons that each shelter was in place (n = 1–6).

Combining habitat and density.—We predicted that occupancy and nesting in artificial shelters would be greatest in reaches that contained limited natural habitat for Hellbenders relative to their densities of adults/subadults (Jachowski et al. 2020). To scale the suitable habitat relative to densities of adult/subadult Hellbenders, we developed a standardized habitat surplus metric by subtracting estimated densities of adult/subadult Hellbenders from large suitable boulder density estimates within each study reach. The surplus values were scaled such that values ranged between 0 and 1.

Two-step analytical approach.—To assess relationships between predictor variables and shelter use by Hellbenders, we adopted a two-step analytical approach from Button et al. (2020). Specifically, we first used permutational multivariate analysis of variance (PERMANOVA) and betadisper analyses to determine whether our predictor variables had a significant collective influence on shelter use. Then, we used boosted regression trees (BRTs) to identify precise relationships between individual predictor variables and shelter use.

Multivariate analyses.—We verified that our set of predictor variables influenced shelter use by Hellbenders using PERMANOVA and betadisper analyses, which determine whether the location and dispersion of points from an ordinated set of predictor variables are related to values of a chosen response variable (i.e., average shelter occupancy/nesting; Dixon 2003). Significant results obtained from PERMANOVA and betadisper analyses provided assurance that any trends reflected actual associations between predictor and response variables (rather than misinterpretations of random differences in the occupancy and nesting use of shelters). Neither PERMANOVA nor betadisper are robust to missing values (Oksanen et al. 2008). Therefore, prior to conducting all multivariate analyses, we used random forest imputations (Stekhoven and Bühlmann 2011) to generate values for missing data (~10% of data used from both datasets, because of limitations imposed on

TABLE 3.—Scores of models predicting occupancy and nesting activity by Hellbenders (*Cryptobranchus alleganiensis*) in artificial shelters deployed within the Upper Tennessee River drainage. Model A = habitat variables from all three spatial scales used during initial model construction; Model B = built with reach-scale predictors excluded during initial model construction; Model C = reach- and core habitat–scale variables excluded during initial model construction; Model D = time since shelter installation and the reach-scale density of Hellbenders as the only predictor variables; Model E = same predictor variables as the top model, but built using data from our six original study locations only. The top performing models are indicated in boldface.

Model	Cross-validated correlation	Cross-validated SE
Occupancy		
A	**0.658**	**0.043**
B	0.655	0.024
C	0.655	0.024
D	0.651	0.049
E	0.649	0.039
Nesting		
A	**0.278**	**0.042**
B	0.205	0.062
C	0.205	0.062
D	0.256	0.047
E	0.215	0.056

data collection by high stream discharges during 2018). We excluded data from reaches sampled for only a single year (hereinafter, "single-year reaches") from both sets of PERMANOVA and betadisper analyses because their inclusion would have required imputing >20% of values for both datasets, given that single-year reaches lacked population density estimates. We standardized all nonbinary predictor variables so that their minimum and maximum values equaled 0 and 1, respectively, and constructed distance matrices for both datasets using Euclidean distances (Lele and Richtsmeier 1991). We performed all multivariate analyses using the vegan package in R (v3.3.3; R Core Development Team 2017).

To visualize the relationships identified by PERMANOVA and betadisper analyses, we generated nonmetric multidimensional scaling (NMDS) plots (Appendix S-II, Supplemental Material). This approach uses distance matrices to collapse data points containing several variables into a specified number of dimensions (Kruskal 1964). We carried out NMDS ordinations for average shelter occupancy and nesting using the minimum number of dimensions where stress was <0.2 (Anderson 2001).

Boosted regression trees.—After verifying that our predictor variables were informative of shelter use via PERMANOVA and betadisper analyses, our second step was to use BRTs to determine the influence of individual predictor variables on occupancy and nesting, using the gbm package in R (v3.3.3; R Core Development Team 2017). BRTs use iterative decision trees to model the influence of predictor variables on a chosen response, and weight each tree based on how much its inclusion in the model minimizes the loss function (Elith et al. 2008). The influence and importance of individual predictor variables is subsequently determined based on their prevalence and average influence across the weighted set of decision trees. Boosted regression trees tend to be useful for identifying ecological thresholds (Elith et al. 2008), and often outperform other modeling approaches (e.g., generalized linear models and generalized additive models) for datasets that are spatially autocorrelated (Crase et al. 2012). We modelled shelter occupancy and nesting activity using separate sets of BRTs, and used average shelter occupancy across all surveys, or average nesting across all breeding seasons, as our unit of replication. Because both response variables had continuous distributions between 0 and 1, we treated them as beta-distributed in all BRT analyses. To account for the differing uncertainty associated with average occupancy and nesting estimates calculated for shelters surveyed or available for nesting differing numbers of times, we assigned shelters weights in our models based on the number of times we surveyed them, or number of breeding seasons they experienced. Specifically, we adopted the approach from Button et al. (2020) to develop a weighting scale that incorporated average occupancy and nesting at shelters, rather than shelter occupancy and nesting during individual shelter surveys, as our unit of replication (Appendix S-III, Supplemental Material).

We included data from all study reaches in our BRTs, including single-year reaches that lacked estimates of population densities of Hellbenders (two on River 1, and two on River 2). Shelter use and habitat data from single-year reaches were valuable even in the absence of density estimates, because we collected these data 5–90 d after shelter installation (n = 318 occupancy and 59 nesting data points), and when differences in shelter occupancy among reaches increased most rapidly (Jachowski et al. 2020). Whereas the inclusion of single-year study reaches in our models did not substantially alter our results, the superior performance of these models corroborated the utility of including single-year reaches in our models (Table 3). This is expected given that BRTs exclude missing values when fitting tree nodes, thus preventing missing data from substantially influencing the shape and slope of modeled relationships. We excluded data from both study reaches in River 1 from our nesting BRT, however, because we were unable to survey these reaches for nests on account of continuously high stream discharge during the 2018 breeding season.

We evaluated BRT performance based on the correlation of model predictions with observed occupancy and nesting values (i.e., cross-validated correlation) using k-fold cross-validation with five folds (Kohavi 1995). We compared models based on their cross-validated predictive performance (Elith et al. 2008). After constructing initial models, we removed variables with <5% contributions, ran these models again, and repeated this process until all variables contributed at least 5% to the model, to avoid overfitting. We also dropped additional variables from our refined models if their inclusion in the model worsened its performance. Based on the cross-validated correlation between predicted and actual response data, we built all models using tree complexity = 2, learning rate = 0.0005, and bag fraction = 0.5, because these values maximized model performance during preliminary model building (Elith et al. 2008). We evaluated the influence of individual predictor variables on shelter occupancy and nesting activity using partial dependence plots (which make predictions by varying a single predictor variable while holding the others constant at their mean) and relative variable influence for predictor variables retained in our top-performing models.

To determine whether including reach-scale variables in our models reduced the estimated influence of finer-scale

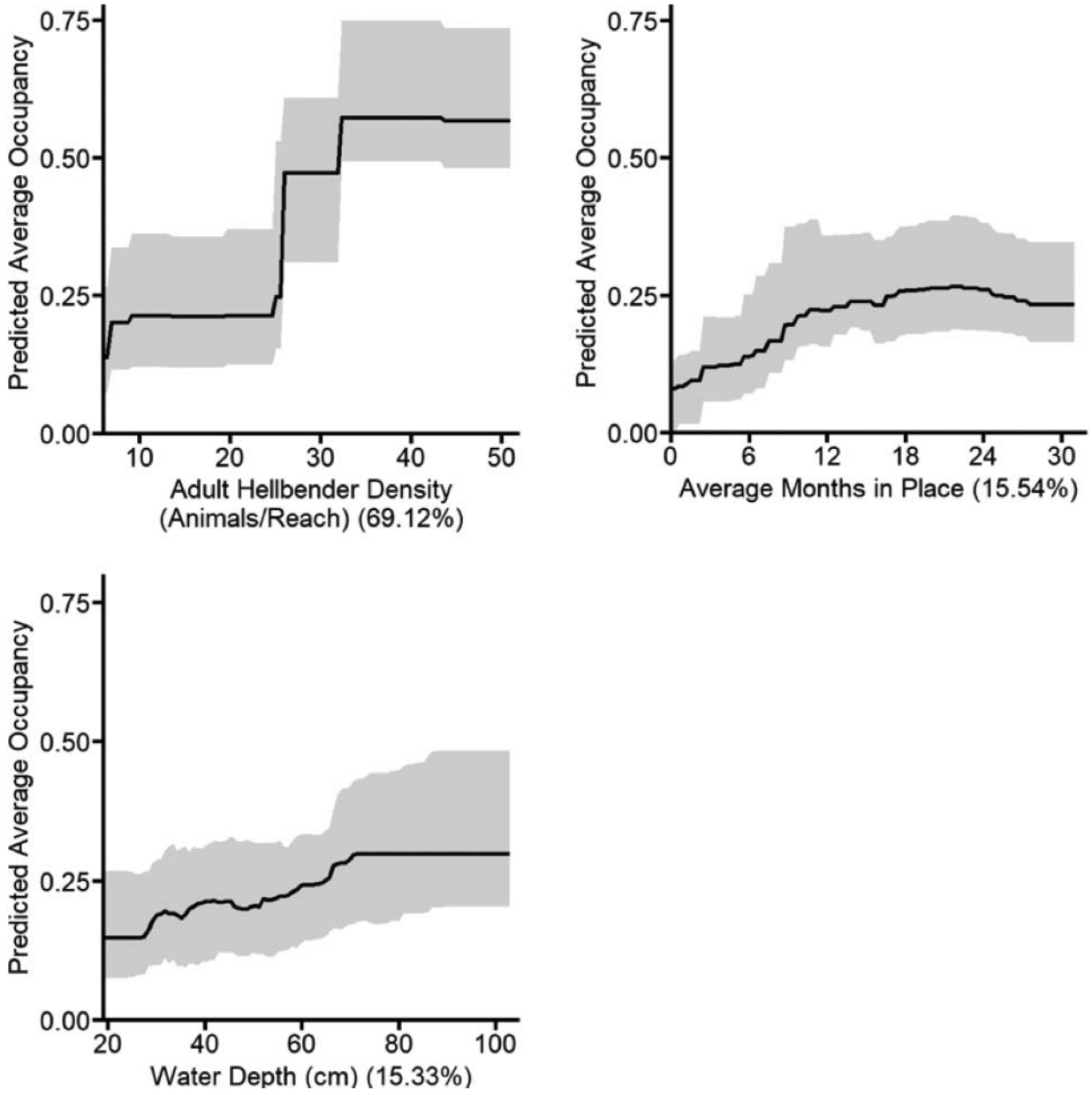

FIG. 1.—Partial dependence plots for the most important predictor variables retained in the final version of the selected model of shelter occupancy by Hellbenders (*Cryptobranchus alleganiensis*) in the Upper Tennessee River drainage. Solid black lines show fitted functions, whereas shaded areas represent 95% percentile–based confidence intervals built using 200 bootstraps. Numbers in parentheses on the x-axes indicate the percentage of influence of each predictor variable on the model's output. Values along the y-axes correspond with predicted average occupancy across all surveys of a given shelter.

habitat variables on shelter use consistent with other studies of resource use by stream-associated species (Thompson et al. 2001; Anderson et al. 2009), we compared the performance of BRTs that excluded 5 m and/or reach-scale predictors to those that included predictor variables from all spatial scales (Appendix S-IV, Supplemental Material). To ensure that the inclusion of data from single-year reaches did not weaken model performance, we also reevaluated the performance of our top model from the set using only data from our six multiyear study reaches with density estimates. Additionally, we reran our top occupancy and nesting model with shelter design as an added predictor variable, to determine whether a tradeoff existed between shelter stability and use.

RESULTS

Whereas Hellbenders did take up residence in the artificial shelters, shelter use varied widely across reaches. In total, Hellbenders occupied artificial shelters on 2518 of 6793 possible occasions (37%), with reach-scale occupancy averaging 22% (range = 0–58%; percentage of occupancy summed across all shelters within a reach) across all surveys. With few exceptions, occupied shelters were used only by adult individuals. Average occupancy peaked at 26% approximately 2 yr after shelter deployment, and remained relatively constant thereafter (Fig. 1). Shelter occupancy increased most rapidly after shelter deployment in reaches containing high densities of adult/subadult Hellbenders

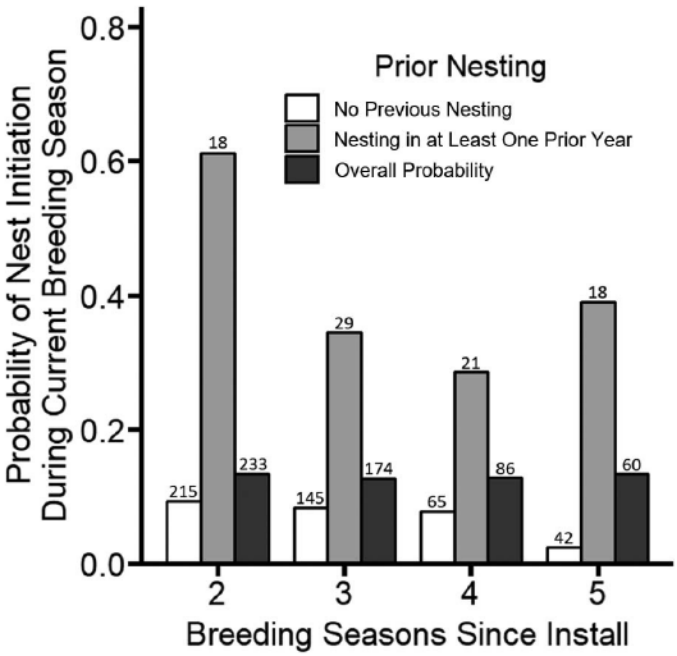

FIG. 2.—The influence of breeding seasons since installation and nesting by Hellbenders (*Cryptobranchus alleganiensis*) during prior breeding seasons on the probability of future nesting activity during a given breeding season, in the Upper Tennessee River drainage. Numbers above bars reflect sample sizes for each group.

(>1.5 individuals/100 m^2), but plateaued after 2 yr regardless of population density (Fig. 1).

Nesting in our artificial shelters by Hellbenders also varied widely across reaches. Hellbenders established nests in shelters on 95 of 925 nesting opportunities (10%; i.e., the summed number of breeding seasons that the shelters were collectively deployed), and reach-scale nest initiation averaged 8% (range = 0–18%) across all breeding seasons. These 95 nests were established in 61 different shelters, by 54 unique males. Prior nesting at a shelter substantially increased the probability of future nesting in that shelter (Fig. 2). Additionally, for shelters in place for multiple breeding seasons, 34% (25/73) that were used for nesting during a given breeding season were also used for nesting by the same male during the previous breeding season. Shelter design exerted <2% influence on our top-performing models when added as a variable to them, indicating that design did not influence occupancy or nesting frequency.

Relationship Between Collective Habitat Variables and Shelter Use

The PERMANOVA and betadisper analyses revealed that the collective variance and average values of our two sets of predictor variables were related to both shelter occupancy ($F = 13.08$, $P = 0.001$, and $F = 4.20$, $P = 0.001$, respectively) and shelter nesting ($F = 2.29$, $P = 0.015$, and $F = 2.38$, $P = 0.016$, respectively). Moreover, whereas our PERMANOVA results should be interpreted with caution on account of the significance of our betadisper results (Oksanen et al. 2008); the betadisper results provided evidence of at least a moderate relationship between shelter use and the dispersion of ordinated predictor variables (Appendix S-II, Supplemental Material; $r = -0.25$ between the response variable and the average distance of predictor variables from the overall centroid for average shelter occupancy, and $r = -0.48$ for average shelter nesting). Therefore, we deemed our two sets of predictor variables appropriate for modeling the relationship between individual predictor variables and shelter use in subsequent BRTs.

Factors Influencing Shelter Occupancy

Our model built using variables from all three spatial scales, prior to dropping unimportant variables, had the highest performance among all models in the set (cross-validated correlation = 0.658, SE = 0.043; Model A in Table 3), and outperformed other models that included predictor variables from two or fewer spatial scales (Table 3; Appendix S-IV, Supplemental Material). The top-performing model indicated that shelter occupancy depended primarily on the density of adult/subadult Hellbenders, and secondarily on average months since installation and water depth at the shelter's tunnel (Fig. 1). Other models in the set performed similarly, and retained a similar set of predictor variables. In the top model, shelter occupancy increased with hellbender density, with that variable being >4 times as influential as any other predictor variable on the model (69% relative influence; Fig. 1). Average months since installation and water depth were also positively associated with shelter occupancy to a lesser degree, and had relative influences of 16% and 15% on the model, respectively. Average shelter occupancy was only positively associated with time since shelter installation during the first 2 yr of shelter deployment, however, and increased most rapidly in reaches with high densities of adult/subadult Hellbenders (Fig. 3). The low contribution of shelter design when added to our top model (0.5%) indicated that it did not influence shelter occupancy. When all variables were optimized, predicted average occupancy reached 67%.

Factors Influencing Shelter Nesting

We constructed our top nesting model (cross-validated correlation = 0.278, SE = 0.042; Model A in Table 3) using predictor variables from all three spatial scales prior to dropping variables with minimal contributions. The top model explained up to 36% more variation in nesting than other models in the set (Table 3; Appendix S-IV, Supplemental Material), and retained water depth (44% influence), population density (34% influence), and the number of breeding seasons (22% influence) as important predictor variables. Predicted nesting frequency was greatest at water depths of approximately 50–60 cm, and increased with increasing densities of adult/subadult Hellbenders (Fig. 4). Average nesting frequency increased during the first three breeding seasons following shelter deployment, and plateaued thereafter. Unlike shelter occupancy, the rate of increase in shelter nesting over time following shelter deployment was unrelated to population density (Fig. 3). The relative influence of shelter design was negligible (2.4%) when added to our top model, suggesting that it did not substantially influence nesting frequency. When all variables were optimized, predicted average nesting frequency reached 24%.

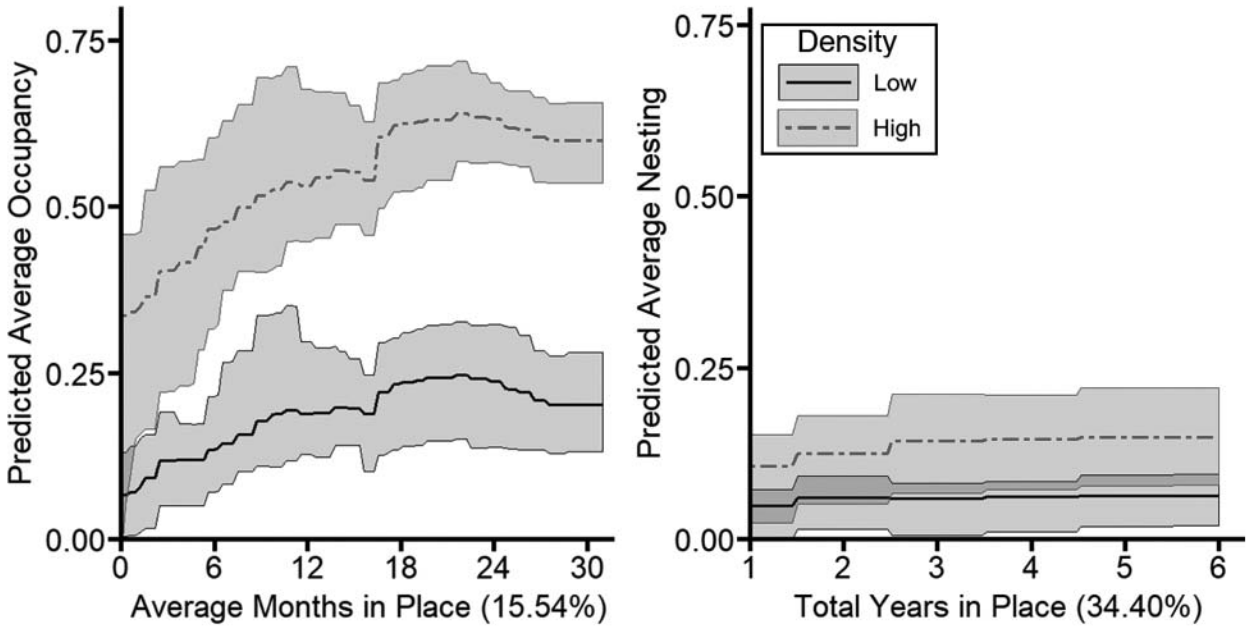

FIG. 3.—The influence of the density of adult/subadult Hellbenders (*Cryptobranchus alleganiensis*) on the relationship between time in place and shelter use in the Upper Tennessee River drainage. Density of Hellbenders was rated as high in reaches with >1.5 adult and subadult Hellbenders per 100 m^2, or otherwise low. Numbers in parentheses on the x-axes indicate the percentage of influence of each predictor variable on the model's output. Values along the y-axes correspond with predicted average occupancy or nesting across all surveys or breeding seasons in which the shelter was deployed.

DISCUSSION

We observed both occupancy and nesting activity by Hellbenders in our artificial shelters, which highlights the utility of these structures for monitoring this sensitive species. Moreover, because we detected an influence of three different variables on occupancy and nesting, only one of which was related to microhabitat features, our results indicate that, within our study system, optimizing shelter placement is a relatively simple process. Given the expected annual occupancy and nesting frequency in optimally placed artificial shelters in this system (up to 67% and 24% respectively), artificial shelters are a potentially powerful tool for monitoring populations of Hellbenders.

Shelter occupancy increased consistently with increasing density of Hellbenders across the reaches examined in our study (Fig. 4), but was influenced by water depth only at the microhabitat scale. Shelter occupancy exceeded 25% in low-density reaches (i.e., <1.0 subadult/adult individual per 100 m^2) within 2 yr of shelter deployment when shelters were deployed in optimal microhabitats (i.e., in portions of the stream >50 cm deep; Table 1). Deployed accordingly, shelter occupancy can improve within reaches where occupancy is otherwise limited by a low density of Hellbenders. This finding suggests that deeply placed artificial shelters might be effective for monitoring Hellbenders across a range of population densities, possibly because Hellbenders are seasonally reliant upon deep runs (Green 1934).

Regardless of where artificial shelters were placed, their occupancy by Hellbenders tended to increase in the first 2 yr following deployment. We attribute this result to the low vagility of Hellbenders (Topping and Peterson 1985; Peterson 1987; Blais 1996; Bodinof et al. 2012), and thus a gradual discovery of the additional habitat provided by artificial shelters. Studies of nest box use by birds and mammals have produced similar results, and have often documented periods of increasing use following artificial shelter installation, which eventually levels off or declines thereafter (McCamant and Bolen 1979; Katzner et al. 2005; Lindenmayer et al. 2009). Given a 2-yr shelter discovery period for Hellbenders, those shelters that were never occupied within 2 yr of deployment (even when deployed in suitable microhabitats) may have gone unused simply because they were not located within the core home range of any individual. Thus, these shelters had low discoverability even when placed in otherwise suitable locations. If the primary objective of shelter deployment is to maximize occupancy of shelters, we recommend relocating shelters that go unoccupied by Hellbenders for two or more consecutive years to improve their likelihood of future occupancy. Given the goal of maximizing shelter occupancy, relocation of shelters might be most beneficial in reaches with high densities of adult/subadult Hellbenders (cf. Fig. 3). Of course, relocating consistently unused shelters requires labor and produces habitat disturbance, so the utility of doing so will depend on project objectives and available resources.

Similar to Jachowski et al. (2020), we found that shelter occupancy increased concomitantly with the density of adult/subadult Hellbenders, and improved for 2 yr following shelter deployment. In contrast to their study, however, we found evidence of a positive relationship between natural shelter density and shelter occupancy by Hellbenders. This discrepancy is most likely attributable to a fundamental difference in how the two efforts assessed the abundance of natural shelters suitable for Hellbenders in a stream reach. Jachowski et al. (2020) considered all boulders (rocks >25.6 cm on the secondary axis) and all bedrock as natural shelter for Hellbenders, and found that boulder/bedrock density was generally negatively associated with artificial shelter occu-

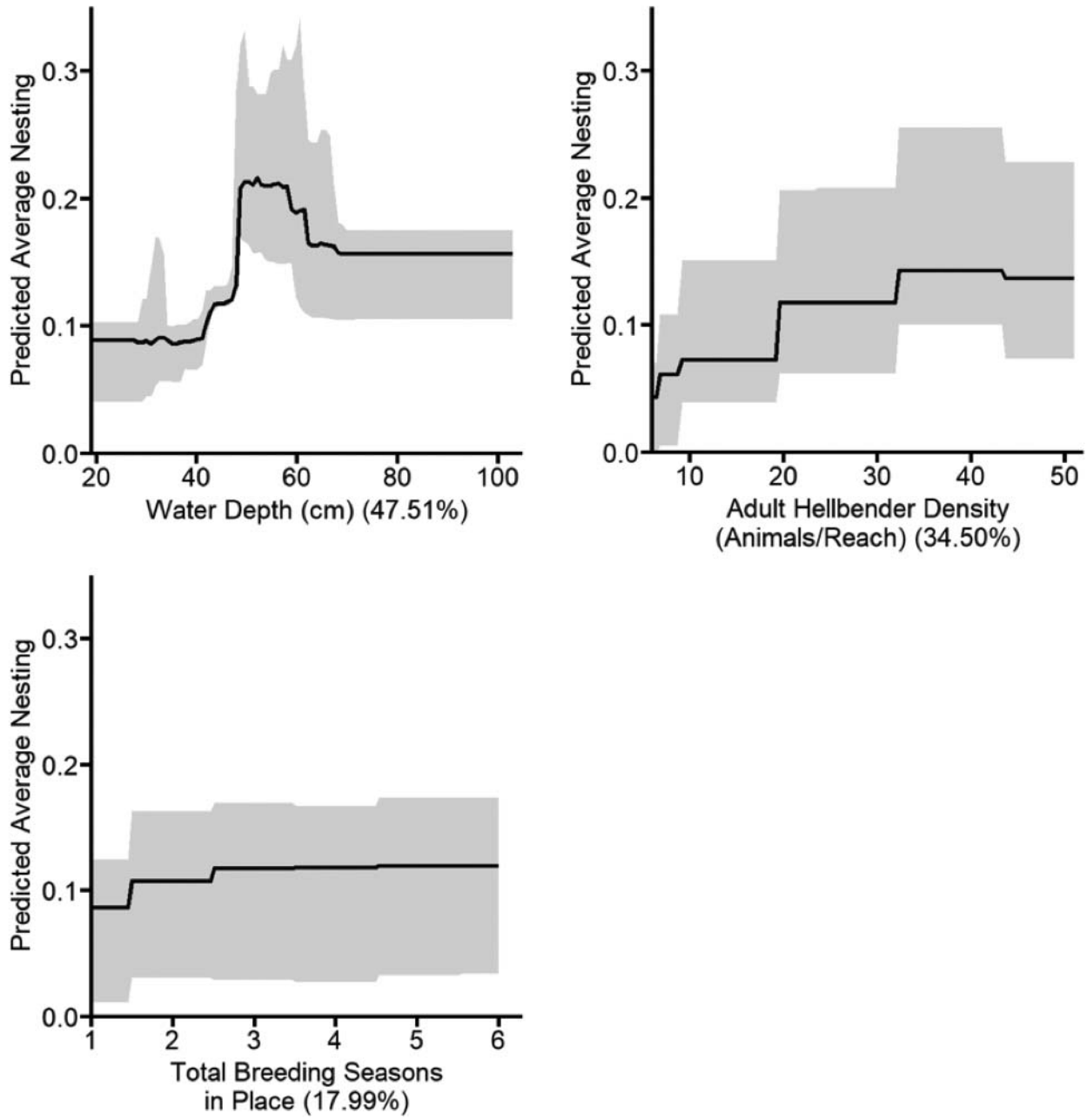

Fig. 4.—Partial dependence plots for the most important predictor variables retained in the final version of the top performing model of shelter nesting by Hellbenders (*Cryptobranchus alleganiensis*) in the Upper Tennessee River drainage. Solid lines show fitted functions, while shaded areas represent 95% percentile-based confidence intervals built using 200 bootstraps. Numbers in parentheses on the x-axes indicate the percentage of influence of each predictor variable on the model's output. Values along the y-axes correspond with predicted average nesting probability across all breeding seasons in which the shelter is deployed.

pancy. In contrast, we only included large boulders (>40 cm on the primary axis) that bore suitable crevices in our estimates of appropriate habitat for Hellbenders. Unlike Jachowski et al. (2020), our metric assumed that all bedrock and boulder lacking suitable crevices were not suitable for Hellbenders. We suspect that our classification of suitable habitat, and possibly other differences in study design (i.e., inclusion/exclusion of different study reaches), explain this particular disparity between the two studies. Additional research is needed to understand the complex interplay among natural habitat availability, population density, and artificial shelter use by Hellbenders.

Nesting activity within shelters was related to similar factors as their occupancy, but the relative influence of these factors was more evenly partitioned (Fig. 4). Nesting was greatest in shelters located in moderately deep (50–60-cm) portions of the stream. Water depth was also nearly three times as important for predicting nesting frequency when compared to breeding seasons since shelter deployment (Fig. 4). Hellbenders might have perceived moderately deep runs as suitable for nesting because these areas featured cooler water than shallower areas (Kramer 1987). Additionally, Hellbenders might have perceived these deeper areas as being better protected from certain predators (e.g., wading

birds) than shallow areas. Because shelter nesting was more than twice as high in moderately deep (50–60-cm) areas than in shallow (20–40-cm-deep) areas, we suggest that monitoring reproduction of Hellbenders will be more successful if shelters are placed in sufficiently deep water.

Based on our results, we provide three practical recommendations for future studies of Hellbenders that incorporate artificial shelters. First, expectations about shelter occupancy and nesting should be scaled according to the reach-wide density of adult/subadult Hellbenders, as shelter use was strongly influenced by this parameter. Secondly, shelters should be deployed in moderately deep locations (at least 50 cm deep in our study system), because doing so improved shelter occupancy and nesting regardless of the density of Hellbenders during our study. Finally, shelters not occupied within 2 yr of installation should be relocated to appropriate microhabitat in another part of the stream channel if the objective is to improve future occupancy, because shelter occupancy did not increase once shelters had already been in place for 2 yr.

Our study is the first to quantitatively evaluate patterns of artificial shelter use by Hellbenders over several (5+) years, and we are encouraged by the occupancy of individual shelters (22%) and the number of constructed nests (95). Habitat features not considered here, or found unimportant in our analyses, might yet be informative for shelter use by Hellbenders outside of our study region. As such, future studies should consider evaluating the applicability of our results to other populations of Hellbenders across the species' range, and assessing whether the relationship between shelter use and other variables not considered here (e.g., density of cobble, alternate shelter design; Mohammed et al. 2016) are equally important for shelter use by Hellbenders. For example, clustering of shelters within habitat patches known to be occupied by Hellbenders is a strategy used in other watersheds to maximize shelter occupancy (J. Briggler, personal communication). Provided that our results are applicable in other watersheds, we suggest that artificial shelters deployed in optimal locations can serve as novel, valuable tools for monitoring and conserving Hellbenders. Our study also serves as a template for using regularly maintained artificial shelters to examine other crevice-associated aquatic species that are secretive and/or of conservation concern (e.g., large crustaceans, certain fish species, and other salamanders).

Supplemental Material

Supplemental material associated with this article can be found online at https://doi.org/10.1655/Herpetologica-D-19-00035.S1.

Acknowledgments.—We thank J. Kleopfer for his invaluable support throughout the study, the landowners who allowed us to access streams through their properties, and B. Brown and J. Snodgrass for their helpful feedback on this manuscript. We also thank J. Hallagan, H. Davie, J. Connock, C. Frazier, M. Lacey, T. Pirault, A. Grimaudo, V. Alaasam, J. Gomes Da Silva Neto, H. Vogel, G. Martin, and S. Beers for their assistance in the field, and help constructing and installing artificial shelters. This research was supported by the Virginia Department of Game and Inland Fisheries, and partially funded through a State Wildlife Grant from the US Fish and Wildlife Service. Additional funding was provided by the US Forest Service and a National Science Foundation grant (IOS-1755055). All protocols were approved under the Virginia Department of Game and Inland Fisheries handling permit (Virginia DGIF Permit No. 060465) and the Virginia Tech Institutional Animal Care and Use Committee (protocols 16-162, 13-128, 11-140, and 08-085).

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Accepted on 22 July 2020
Associate Editor: Michael Freake