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Distribution and Abundance of Introduced Seal Salamanders (Desmognathus monticola) in Northwest Arkansas, USA

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Distribution and Abundance of Introduced Seal Salamanders (*Desmognathus monticola*) in Northwest Arkansas, USA

Clint L. Bush[1], Jacquelyn C. Guzy[1], Kelly M. Halloran[1], Meredith C. Swartwout[1], Chelsea S. Kross[1], and John D. Willson[1]

Many reptiles and amphibians are gaining recognition as harmful invaders, highlighted by well-known examples such as the Brown Tree Snake (*Boiga irregularis*), Cane Toad (*Rhinella marina*), American Bullfrog (*Lithobates catesbeianus*), and Burmese Python (*Python molurus bivittatus*). In 2003, an introduced population of Seal Salamanders (*Desmognathus monticola*) was found in Spavinaw Creek, within the Ozark Plateau of northwest Arkansas. Genetic evidence confirmed an introduction from northern Georgia. Very little is known about the status of this non-native population; thus, the objective of this study was to assess the current distribution and abundance of non-native *D. monticola* along Spavinaw Creek. We conducted repeated, low-intensity visual surveys along the 30 km extent of Spavinaw Creek in Arkansas and used a hierarchical Bayesian analysis to model the occupancy response of *D. monticola* and five native salamander species relative to river mile and habitat covariates. We also conducted a short-term closed capture-mark-recapture study to estimate abundance of *D. monticola* at the original collection site on Spavinaw Creek. We found a clear geographic pattern of distribution of *D. monticola*, with individuals found throughout the upper 10 km of Spavinaw Creek headwaters, but no clear habitat associations. Estimated abundance of *D. monticola* was extremely high—14.5 individuals and 50 g wet biomass per m². Our results reveal that introduced *D. monticola* are much more widely distributed than previously recognized and occur at high densities, suggesting that this recent invader could negatively affect ecosystems of Spavinaw Creek and surrounding watersheds in the Ozark highlands.

REPTILES and amphibians are now recognized for their ability to become harmful invaders (Kraus, 2015). Lowe et al. (2004) include five reptile and amphibian species in their list of the 24 most damaging invasive vertebrates. Particularly well-known invasive reptiles and amphibians include the Coquí frog (*Eleutherodactylus coqui*), Cane Toad (*Rhinella marina*), American Bullfrog (*Lithobates catesbeianus*), Brown Tree Snake (*Boiga irregularis*), and Burmese Python (*Python molurus bivittatus*). Although non-native herpetofauna are occasionally intentionally released as food sources and bio-controls, reptiles and amphibians have several characteristics that may predispose them to unintentional introduction via the pet trade, as accidental stowaways in shipping containers, and as hitchhikers on agricultural products (Pitt et al., 2005). In particular, small size and cryptic coloration allow them to avoid detection, and their popularity in the pet trade opens up frequent opportunities for their introduction to non-native areas. Many invasive reptiles and amphibians are generalist feeders and have high reproductive potential, allowing them to rapidly attain large population sizes. As a result, invasive herpetofauna can pose multiple threats to native species via alterations to trophic dynamics and competition with native species, among many other mechanisms (Kraus, 2015). Because many reptiles and amphibians are semi-aquatic, they pose a particular threat to aquatic systems.

Streams are considered to be among the most ecologically valuable aquatic habitats (Allan and Castillo, 2007; Meyer et al., 2007), but are also known to be sensitive to biological invasion (Mooney and Cleland, 2001). Because of their hydrological connectivity, ability to sequester nutrients and matter from surrounding watersheds, heterogeneity of habitat composition, and environmental and regional differences, freshwater ecosystems, including streams, are hotspots of biodiversity (Smith et al., 2002; Abell et al., 2008). Ecosystem services provided by streams include mitigation of drought and flooding, detoxification and decomposition of waste, and the maintenance of biodiversity (Meyer et al., 2003). Notably, invasive species are one of the five primary threats to freshwater biodiversity (Dudgeon et al., 2006). Streams frequently receive invasive species through fish stocking for sport or biocontrol, fishing bait and aquaria releases, and deposition of hitchhiking species from other aquatic habitats (Shireman, 1984; Rupp, 1996; Casal, 2006; Moyle and Marchetti, 2006). Given this, invasive species have the potential to dramatically alter stream ecosystems and extirpate native species. For example, in New Zealand streams, the introduction of Brown Trout (*Salmo trutta*) caused trophic cascades that resulted in increased algal biomass and changes to energy and nutrient flux (Simon and Townsend, 2003).

In 2003, an introduced population of Seal Salamanders (*Desmognathus monticola*) was discovered in Spavinaw Creek, in the Ozark Mountains of extreme northwestern Arkansas (Trauth et al., 2004; Bonett et al., 2007). Genetic evidence traced the source of the introduction to northern Georgia (Bonett et al., 2007). The Ozark Plateau is one of the North American hotspots of stream biodiversity and endemism (TNC, 2003). This region shares many similarities with the native habitat of *D. monticola*, which occur naturally in small, cool, well-aerated streams along the Appalachian Mountains from southwestern Pennsylvania to northern Georgia, with isolated populations in southern Alabama and the Florida Panhandle (Petranka, 1998). Although the Ozarks and the southern Appalachians are both dominated by deciduous forests with abundant headwater streams, rocky substrates, and high native stream biodiversity, there are no native *Desmognathus* salamanders in the Ozark highlands (Trauth et al., 2004). Previous studies have shown recruitment in the population of *D. monticola*, confirming a breeding population in Spavinaw Creek (Bonett et al., 2007; Connior et al., 2013). However, nothing is currently known about the

distribution or abundance of *D. monticola* in northwest Arkansas, making it difficult to assess their status or potential threat to native species.

The primary objective of this study was to assess the current distribution and abundance of non-native *D. monticola* along Spavinaw Creek, Benton County, Arkansas. We used repeated low-intensity visual surveys at 27 sites along the extent of Spavinaw Creek within Arkansas to map the distribution of the species. We also conducted a short-term capture-mark-recapture study to estimate abundance of *D. monticola* at the original collection site on Spavinaw Creek. Our results provide a first step in assessing the invasion status and potential impacts of this novel invader.

MATERIALS AND METHODS

Study sites.—The first goal of our research was to quantify distribution of introduced *D. monticola* in suitable habitat along the Arkansas portion of Spavinaw Creek, from the Oklahoma border east to the headwaters. Spavinaw Creek is a groundwater-fed stream surrounded by a mosaic of agriculture and deciduous forest overlaying karst topography with headwaters in central Benton County, in the extreme northwest corner of Arkansas (Fig. 1). It flows west-southwest, crossing the Oklahoma border and draining into the Grand River, and eventually, the Arkansas River. We conducted low-intensity presence-absence surveys at a total of 27 sites spaced approximately 1 km apart along the entire reach of Spavinaw Creek in Arkansas (Fig. 1). Sites varied in elevation between 294.14 m and 389.17 m (mean 334.45; SD 28.88). Because a majority of the properties bordering Spavinaw Creek are privately owned, we used a county-specific geographic information system record to identify property boundaries and ownership. As a result of limited access, some gaps between sites exceeded the target spacing of 1 km. Once permission was granted by landowners, we visited properties and selected specific sampling sites that contained suitable habitat for salamanders. Preferable site characteristics included: near or at a spring or first order tributary, north facing slope, forested cover, exposed bedrock, shallow water, steep banks, and burrows in banks.

Occupancy sampling.—At each of the 27 sites, a single observer (CLB) conducted three low-intensity presence-absence surveys to quantify distribution of *D. monticola* and other native salamander species, while accounting for imperfect detection. Surveys were conducted between 31 March and 29 June 2015, with an average of 18 days between successive surveys at each site. To maximize detectability of salamanders on the surface, surveys were conducted at night when air temperature was greater than or equal to 7.2°C (Hyde and Simons, 2001; Connette and Semlitsch, 2012). Each survey consisted of a 10 min visual encounter survey along a 10 m linear stretch of creek. The observer searched both banks, including the shallow water's edge whenever flow rate was low. We also measured several potential sampling covariates at each survey including date, time, relative humidity, barometric pressure, current precipitation, number of days since last rain, and whether the stream was near base-flow conditions. Site covariates measured in the field for each site included stream width, a percentage-based substrate composition score for four size classes of substrate (%boulder/cobble, %gravel/fine gravel, %sand/silt, and %bedrock) based on size categories determined by the

Wentworth scale of grain size (Wentworth, 1922), and a bank burrow presence score within a four-point rating system (0 = no burrows, 1 = 1–5 burrows observed, 2 = 6–10 burrows, and 3 = >10 burrows).

When possible, we captured all salamanders and placed them in a clear plastic container for the duration of the survey to avoid double-counting individuals. At the end of the survey, we identified salamanders to species, photographed them, and released them back to the original sampling site. At locations where there were very high numbers of salamanders, we simply identified and counted them to avoid wasting sampling time. Three independent reviewers checked photos to validate salamander identification. Due to difficulties differentiating larval *Eurycea longicauda* and *E. lucifuga* from photographs, these were excluded from analyses ($n = 7$ individuals).

Landscape data collection and data analysis.—We measured a series of variables for our study site by building a geographical information system (ArcGIS 10.2.2 ESRI, Redlands, CA) based on georeferenced digital orthoimagery obtained from the U.S. Department of Agriculture seamless server (https://gdg.sc.egov.usda.gov/GDGOrder.aspx) and stream order classification, elevation, landcover, slope, and data from the Arkansas GIS Office server (http://gis.arkansas.gov/). These variables included the river distance from the Oklahoma border ('River Mile'), area of forest and average elevation, slope, aspect, roughness (i.e., variance in the elevation), and moisture index (Iverson et al., 1997) within a 250 m buffer of each study site. Geographical data were calculated using the 'zonal statistics as table' tool in combination with the 'geomorphology and gradient metrics' toolbox (Evans et al., 2014) and 'slope' and 'aspect' tools in ArcGIS. We selected a radius of 250 m around each sampling location because this distance is large enough to provide information at a landscape scale but is small enough to provide spatial independence (i.e., non-overlapping circular buffers).

We used principal components analysis (PCA) in PRIMER 6.0 (Clarke and Gorley, 2006) to reduce potentially correlated site variables to a lower number of uncorrelated components (Clarke, 1993). We performed a PCA on both geography variables (i.e., percent of forest, slope, aspect, roughness, and moisture index) and microhabitat variables (i.e., percent of boulders/cobble, percent of bedrock, and bank burrow index). Because we had a relatively small sample size, our ability to include multiple covariates was constrained; thus, we only used the principal component scores from each of the first PC axes as site covariates in our occupancy analyses.

Occupancy analyses.—We used a multi-species hierarchical Bayesian model to estimate species-specific occupancy responses to three site-specific covariates (River Mile, Geography PC1, and Microhabitat PC1). More specifically, we implemented the model used by Hunt et al. (2013) and modified from Zipkin et al. (2009); this hierarchical approach treats species-specific mean occupancy and detection responses to covariates as originating from an assemblage-level (i.e., all salamander species together) distribution, and thereby estimates both species-specific and assemblage-level responses in the same modeling framework (Dorazio and Royle, 2005; Zipkin et al., 2009).

Our analysis accounted for imperfect detection of individual species through repeat surveys; we therefore did not assume all species are present at every site or that non-detection reflects species absence (Dorazio and Royle, 2005;
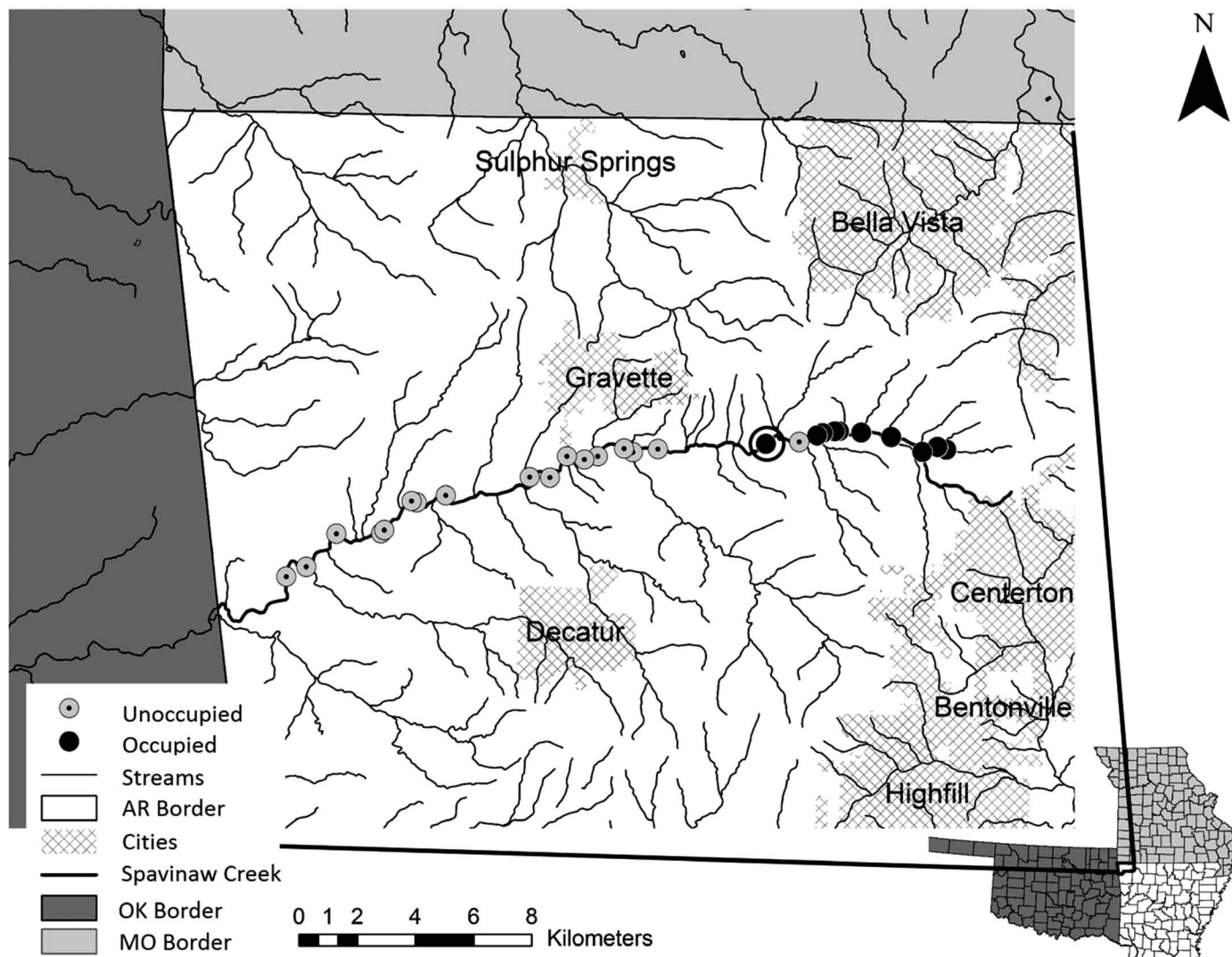
Fig. 1. Map of study locations sampled to investigate distribution and abundance of non-native Seal Salamanders (*Desmognathus monticola*) along Spavinaw Creek, Benton County, northwest Arkansas, USA, relative to adjacent regions of Oklahoma and Missouri. Inset shows the location of each occupancy sampling site along Spavinaw Creek, with filled circles indicating sites found to be occupied by *D. monticola*. Sites are numbered (Fig. 2) sequentially from west to east. The location of the capture-mark-recapture site (site 17) is indicated by the hollow black circle.

Dorazio et al., 2006; Kéry et al., 2009). Furthermore, in a hierarchical analysis, individual parameter estimates, particularly for rare species, are improved (made more precise and less likely to be biased) by considering them in the context of the larger community (Sauer and Link, 2002; Zipkin et al., 2009).

We used the following equations to relate species-specific occupancy and detection probabilities (Ψ_{ij} and Θ_{ijk}, respectively) to our model. More specifically, i, j, and k represent species, site, and sampling occasion, respectively, α is the site-level effects on occurrence, and u_i and v_i are species-specific intercepts:

$$\text{logit}(\Psi_{ij}) = u_i \alpha 1_i River\,Mile_j + \alpha 2_i Geography\,PC1_j \\ + \alpha 3_i Microhabitat\,PC1_j$$

$$\text{logit}(\Theta_{ijk}) = v_i$$

We defined the *River Mile* covariate as the z-score of distance upstream from the Oklahoma border for all survey locations (i.e., site's distance–mean/SD). We defined the

Geography PC1 and *Microhabitat PC1* covariates as the respective z-score of PCA-1 values for geography and microhabitat variables for each sampling location. Standardized covariates allowed us to estimate Ψ and Θ at mean site and survey covariates (where the z-score equals zero) from model-generated estimates of u_i and v_i, and allowed direct comparison of model coefficients as effect sizes relative to variation in each covariate.

The model therefore contained the following parameters, specific to each species: u_i, $\alpha 1_i$, $\alpha 2_i$, $\alpha 3_i$, v_i. Our model used uninformative priors for all μ parameters (e.g., U[–3 to 3]); species-specific model coefficients were truncated at ±5 from μ to avoid traps. The use of vague priors is suited to ecological applications because this reflects a lack of prior knowledge of a parameter's true value (Link et al., 2002). The mean and standard deviation of the model coefficients were calculated, along with the 2.5 and 97.5 percentiles of the distribution, which represent a 95% Bayesian credible interval (CI). We inferred significance for continuous covariates when CIs did not contain zero. Species-specific occupancy and detection

estimates were derived using the inverse logit transformation (i.e., exp [α]/[1+ exp α]).

We organized our data in program R (2.14.0; R Development Core Team, 2011) and executed data analysis in the software program WinBUGS (Lunn et al., 2000) using R2WinBUGS (Sturtz et al., 2005). The model was run on three independent chains of 300,000 iterations each, after a burn-in period of 30,000 iterations. Output was thinned by a factor of three, so inference was based upon 300,000 samples from the stationary posterior distribution. Evidence for lack of convergence was assessed by examining history plots and the Gelman and Rubin statistic (<1.02 for all monitored parameters; Gelman and Rubin, 1992).

Capture-mark-recapture sampling.—To assess abundance of *D. monticola* within its introduced range, we conducted a short-term capture-mark-recapture (CMR) study at the original collection location on Spavinaw Creek (Fig. 1; Bonett et al., 2007). This site consists of a small springhead that flows approximately 10 m across relatively level terrain from a dirt hillside into the main channel of Spavinaw Creek. We conducted six surveys of a 10 × 3 m stream-side transect over a three-month period in fall 2014 (8 August; 3, 12, 18, September; 2, 8 October). Surveys consisted of four observers sampling for two hours between 2000 and 2300 hours. We searched all available cover objects in the stream and captured salamanders with dip nets. In addition, we extracted salamanders from bankside burrows by guiding a length of coat-hanger wire into the burrow and gently tapping the posterior end of the salamander. After each survey, we returned all captured *D. monticola* to the laboratory, measured them for SVL and mass, and marked them individually using subcutaneous injection of visible implant elastomer (VIE; Northwest Marine Technologies, Shaw Island, WA; Marold, 2001). Visual examination of size frequency distribution of captures revealed a clear break in the size distribution at 40 mm SVL, with smaller individuals representing individuals that metamorphosed the previous summer. Preliminary CMR analyses indicated that these individuals differed in capture probability from older individuals. Thus, each animal was assigned an age class of 1st year juvenile (i.e., ≤40 mm SVL) or adult (>40 mm SVL). These categories represent age classes, not necessarily sexual maturity, which is unknown in this population. We individually marked each salamander at three of six possible locations along the venter, corresponding to each of the four legs and either side of the cloaca. To administer marks, we anesthetized salamanders in a solution of 1.0 g/L of maximum strength Orajel® (Cecala et al., 2007). We released salamanders in the evening, within three days of initial capture.

Capture-mark-recapture analyses.—We used our record of captures and recaptures over six surveys to estimate abundance of *D. monticola* by applying five models designed for estimation of closed populations in program MARK (v. 7.1; White and Burnham, 1999), using the 'full likelihood p and c' data type. This category of models includes estimation of abundance (N) in the likelihood expression (Otis et al., 1978). In addition to the null (constant and equal capture [p] and recapture [c] probability) model 1) [p(.) = c(.)], we applied four other models to the dataset, each accounting for a different potential source of variation in p and c probability: behavioral effects, model 2) [p(.), c(.)]; age variation, no behavioral effects, model 3) [p(age) = c(age)]; time variation,

no behavioral effects, model 4) [p(time) = c(time)]; age and time variation, no behavioral effects, model 5) [p(age*time) = c(age*time)]. Although we initially applied additional models accounting for a combination of the above factors, we do not present the results here because these models frequently yielded nonsensical parameter estimates, likely as a consequence of our relatively small sample size.

To select the model that best fit our data, we used Akaike Information Criteria (Akaike, 1973) adjusted for small sample size (AICc; Burnham and Anderson, 2002). We tested model goodness-of-fit for our most parameterized model by using the median ĉ procedure implemented in program MARK (White and Burnham, 1999), which calculated a variance inflation factor (ĉ) of 1.41 for our data. We selected best models using lowest model weights along with quasi-likelihood-adjusted Akaike Information Criterion adjusted for small sample size (QAICc; Burnham and Anderson, 2002).

RESULTS

Occupancy.—The first geography site-covariate PCA axis explained 63.1% of the variance (Table 1A). Geography PC1 had relatively high factor loadings for increasing elevation and moisture index and decreasing slope and roughness index; thus, we consider Geography PC1 to be a general index of topographic wetness. The first microhabitat site-covariate PCA axis explained 64.1% of the variance (Table 1B). Microhabitat PC1 had relatively high factor loadings for decreasing percentage of rocks and cobble, and increasing bedrock and amount of bank burrows; thus, we consider Microhabitat PC1 to be a general index of substrate/bank habitat quality.
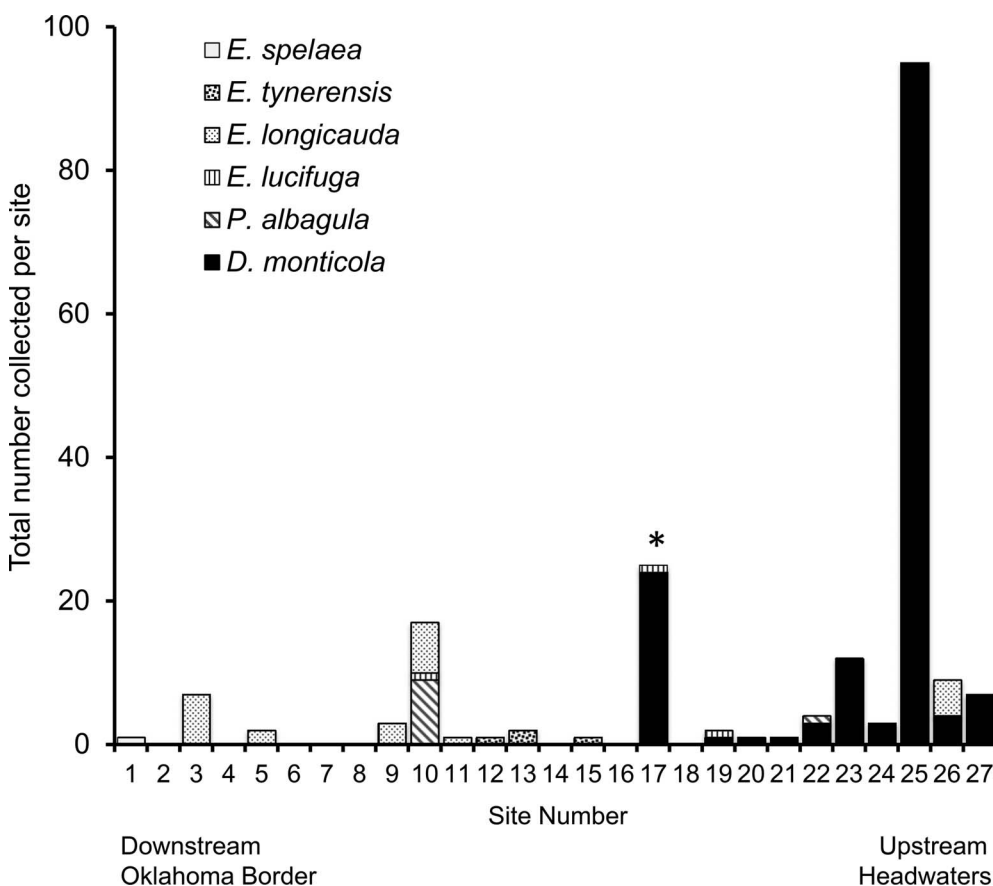
Repeated, low-intensity occupancy sampling of 27 locations along Spavinaw Creek yielded six salamander species: the non-native Seal Salamander (*Desmognathus monticola*, n = 150), and native Oklahoma Salamander (*Eurycea tynerensis*, n = 4), Long-tailed Salamander (*E. longicauda*, n = 27), Cave Salamander (*E. lucifuga*, n = 4), Grotto Salamander (*E. spelaea*, n = 1), and Western Slimy Salamander (*Plethodon albagula*, n = 9; Fig. 2). Total numbers of salamanders captured over three occupancy surveys ranged from 0 to 95 (Fig. 2), with *D. monticola* captured at most of the upstream (easterly) sites. When present, *D. monticola* were often found at high abundances, with one site yielding 95 *D. monticola* over the three 10 min surveys (Fig. 2). Conversely, native salamanders were usually not found at high abundances and most captures occurred in the middle and downstream sections of the creek.

Our model indicated mean estimated species detection probabilities were variable among species (0.13–0.63) with mean detection estimates of 0.63 (95% CI 0.45–0.79) for *D. monticola*, 0.63 (95% CI 0.36–0.85) for *E. longicauda*, and 0.38 (95% CI 0.09–0.85) for *P. albagula* (Fig. 3). Conversely, mean occupancy probabilities among species were similar (0.16–0.21) with mean occupancy of *D. monticola* estimated at 0.19 (95% CI 0.08–0.41; Fig. 3). Occupancy of *D. monticola* was strongly associated with increasing distance upstream from the Oklahoma border (i.e., River Mile; Table 2); occupancy probability varied from 0.002 (95% CI 0.00–0.005) at the border to 0.86 (0.63–0.97) at a distance 33 km upstream from the Oklahoma border (Fig. 4). A threshold was evident in these data, indicating very low probability of occupancy of *D. monticola* near the Oklahoma border, and probability of occupancy rising rapidly by 15 km upstream of the border (Fig. 4). Remaining species showed no relationship with River

Table 1. Character loading and percentage of variance explained by (A) principal components (PC) axis 1 for six geographic variables, and (B) principal components (PC) axis 1 for three microhabitat variables expected to influence salamander occupancy at Spavinaw Creek. Bold figures indicate variables with the highest loadings.

Variable	PC 1
A)	
Eigenvalues	3.780
% Variation	63.1
Cum. % Variation	63.1
% Forest	−0.360
Elevation	**0.450**
Slope	**−0.468**
Aspect	0.199
Roughness index	**−0.477**
Moisture index	**0.426**
B)	
Eigenvalues	1.920
% Variation	64.1
Cum. % Variation	64.1
% Boulders, cobble	**−0.648**
% Bedrock	**0.589**
Amount of bank burrows	**0.482**

Mile (95% credible intervals for parameter estimates contained zero; Table 2). We observed a positive mean occupancy response for *E. longicauda* and *E. lucifuga* with increasing Microhabitat PC1 score; occupancy increased with decreasing amount of cobble and increasing amount of both bedrock and bank burrows (Fig. 5). Remaining species showed no relationship with either Geography PC1 or Microhabitat PC1 (Table 2).

Abundance.—Six mark-recapture sampling events at a single 10 × 3 m location (site 17; Figs. 1, 2) yielded 401 captures of 249 individual *D. monticola* (Fig. 6). The number of recaptures per sampling event ranged from 8 to 44 and the overall proportion of recaptures was 0.38 (Fig. 6). The best supported model included age- and time-specific capture/recapture probability (QAICc $w = 1.00$; Table 3), with no support for behavioral responses. Individual capture probability varied from 0.19 (95% CI 0.12–0.29) to 0.40 (0.30–0.50) across sampling events for 1st year juvenile *D. monticola* and from 0.003 (0.00–0.03) to 0.20 (0.13–0.31) across sampling events for adults. The number of 1st year juvenile and adult *D. monticola* at our mark-recapture sampling location was estimated to be 129 (95% CI 117–141) and 307 (203–412), respectively, which corresponds to an estimated density of approximately 436 *D. monticola* within the 30 m² sampling area (14.5 per m²). Taking into account the mean masses for 1st year juvenile (0.58 g) and adult (4.65 g) *D. monticola* at the site, this corresponds to a mean wet biomass of 50 g per m².

DISCUSSION

In 2003, a small but apparently thriving introduced population of *Desmognathus monticola* was discovered in a small spring that flows into Spavinaw Creek, Benton County, Arkansas (Trauth et al., 2004; Bonett et al., 2007). Their presence was reconfirmed at this site in 2012 by the capture of more than ten individuals by Connior et al. (2013). Another locality was subsequently found at a small spring 2.5 km upstream of the original site (Bonett et al., 2007), but Connior et al. (2013) failed to find any *D. monticola* at this site. We found *D. monticola* at both previously recorded
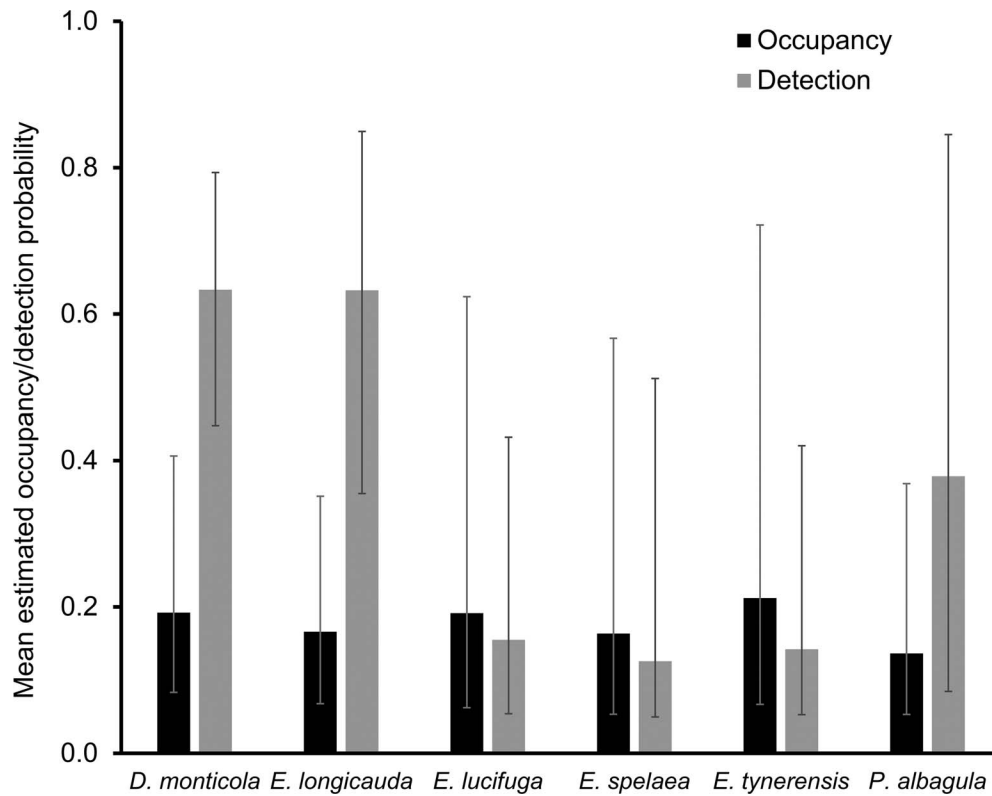
Fig. 2. Salamanders captured by species and sampling site during low-intensity occupancy surveys along Spavinaw Creek, northwest Arkansas. Captures represent totals over three 10 min nocturnal visual surveys per site. An asterisk (*) denotes the site where density was estimated via capture-mark-recapture.

Fig. 3. Estimated probability of occupancy (black bars) and detection (gray bars) for salamander species along Spavinaw Creek, Benton County, Arkansas, USA. Estimates are based on three 10 min nocturnal visual surveys per site. Error bars represent 95% credible intervals.

locations, and at nine out of ten upstream sites, as far as 7 km upstream of the original collection location. Although we did not sample the extreme headwaters of Spavinaw Creek (Fig. 1), it is probable that *D. monticola* occur there as well. Species detection probability of *D. monticola* was high enough that we are confident populations are not established along the main channel of Spavinaw Creek within approximately 15 km of the Oklahoma border (Figs. 1, 4). Our study reveals that *D. monticola* are more widely distributed along Spavinaw Creek than previously thought and occur at extremely high densities at some locations. The Ozark Plateau lacks any native large-bodied stream salamanders; thus, *D. monticola*

may be highly successful and potentially harmful invaders exploiting a vacant niche.

Although the exact location of introduction is unknown, there are at least three possible scenarios that could explain the distribution of *D. monticola* in Spavinaw Creek (Fig. 1): 1) the sites of introduction are those reported by Bonett et al. (2007) and Connior et al. (2013) and the salamanders are preferentially spreading upstream; 2) the site of introduction is in the headwaters and salamanders are spreading downstream; or 3) regardless of introduction origin, the salamanders have dispersed throughout suitable habitat, avoiding downstream reaches because the habitat is unsuitable.

Table 2. Summary of occupancy covariate parameter estimates and 95% credible intervals for salamander species along Spavinaw Creek, Benton County, Arkansas, USA. Bold values indicate statistical significance (i.e., credible intervals do not contain zero, or the majority of the CI distribution is positive or negative).

| Species	Parameter	Parameter estimate	2.5% CI	97.5% CI
Desmognathus monticola	**River Mile**	**2.532**	**1.56**	**2.99**
	Geography PC1	−0.004	−0.51	0.58
	Microhabitat PC1	0.116	−0.81	0.94
Eurycea longicauda	River Mile	−0.352	−1.78	0.85
	Geography PC1	−0.267	−0.96	0.25
	Microhabitat PC1	**0.930**	**0.10**	**2.11**
Eurycea lucifuga	River Mile	1.187	−0.84	2.85
	Geography PC1	−0.205	−1.15	0.49
	Microhabitat PC1	**1.087**	**−0.08**	**2.62**
Eurycea spelaea	River Mile	−0.726	−2.70	1.31
	Geography PC1	−0.060	−1.23	1.00
	Microhabitat PC1	−0.456	−2.44	1.07
Eurycea tynerensis	River Mile	−0.169	−2.61	2.26
	Geography PC1	−0.163	−1.09	0.54
	Microhabitat PC1	−0.928	−2.80	0.69
Plethodon albagula	River Mile	0.510	−1.14	2.44
	Geography PC1	0.190	−0.44	1.32
	Microhabitat PC1	0.437	−0.51	1.55
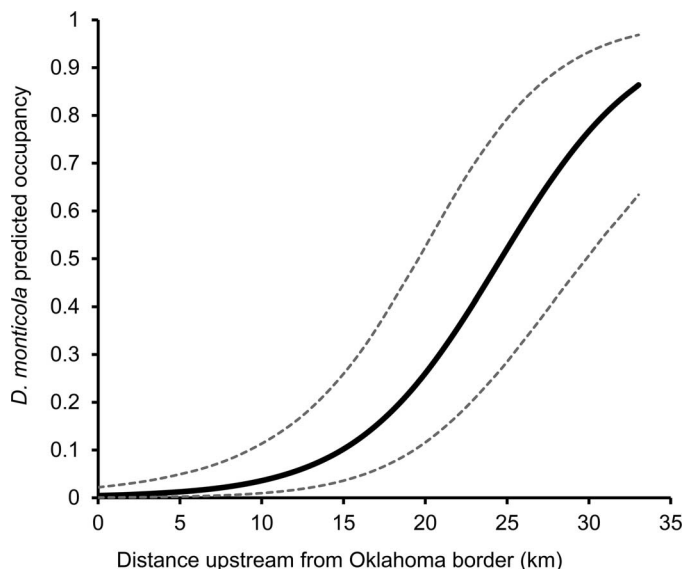
Fig. 4. Effect of distance upstream of Oklahoma border on occupancy probability of *D. monticola* along Spavinaw Creek, Arkansas, USA. Solid line represents the mean relationship between distance and occupancy probability and dashed lines are the 95% credible intervals for estimates of the covariate effect.

Lending support to Scenario 1, the original collection location is one of the few publicly accessible road crossings on Spavinaw Creek. Many stream salamanders (i.e., *Gyrinophilus, Desmognathus*) exhibit upstream-biased dispersal, possibly due to a preference for habitat at stream headwaters or as a way to compensate for downstream displacement during high flow (Lowe et al., 2006; Grant et al., 2010). In addition, during reproduction, female *Desmognathus* preferentially seek out shallow upstream sections of headwater streams that are free from large predatory fishes (Snodgrass et al., 2007). However, if Scenario 2 is true, time may be the primary factor limiting dispersal of *D. monticola*—and in this
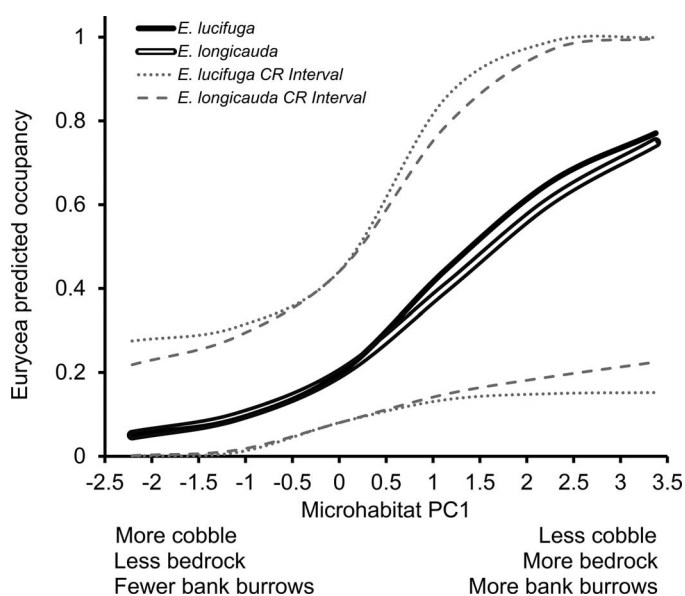
Fig. 5. Effect of microhabitat PC1 on occupancy probability of *E. lucifuga* and *E. longicauda* along Spavinaw Creek. Solid lines represent the mean relationship between PC1 scores and occupancy probability and dashed lines are the 95% credible intervals for estimates of the covariate effect.
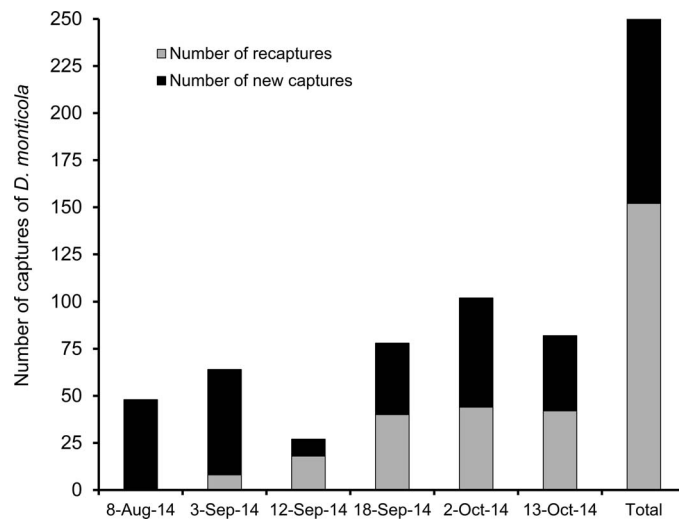
Fig. 6. Number of new captures and recaptures of individual *D. monticola* per survey at 10 m x 3 m mark-recapture site on Spavinaw Creek, Arkansas.

scenario, further downstream spread of *D. monticola* over time is likely. Under Scenario 3, distribution of *D. monticola* corresponds to the availability of suitable habitat in Spavinaw Creek. *Desmognathus monticola* can be found in a variety of streamside habitats, but individuals do have a preference for larger rocks (Southerland, 1986a). Our study did not find any strong association of occupancy of *D. monticola* with availability of large rocks, burrows, substrate composition, or stream width (Table 2), suggesting that habitat structure is not limiting dispersal. However, these results could be misleading if salamander reproduction only occurs in more favorable habitats, such as headwater streams, and then larvae and juveniles disperse throughout other sections of the creek. Further research on demography and dispersal patterns of this introduced population are needed to elucidate which scenario is most probable.

Even if Scenario 3 is true and availability of suitable habitat limits the spread of *D. monticola* beyond its current distribution in Spavinaw Creek, they may still be able to colonize nearby watersheds through overland dispersal. The headwaters of Spavinaw Creek are adjacent to Little Sugar Creek in the Elk River drainage, and tributaries of Little Sugar Creek flow within 5 km of documented localities of *D. monticola*. *Desmognathus monticola* have been documented moving up to 8.5 m away from streams and thus they may be able to use forested corridors and first order streams to disperse between Spavinaw Creek and adjacent watersheds (Crawford and Semlitsch, 2007). Alternatively, salamanders could also easily be transported between watersheds through their use as fishing bait or as stowaways in river gravel.

In addition to demonstrating that *D. monticola* are now widespread in the upper reaches of Spavinaw Creek, our study also revealed that they can exist at extremely high densities. Our capture-mark-recapture analysis yielded an estimated density of 14.5 individuals per m^2, amounting to a wet biomass of 50 g/m^2. Although density of stream-associated salamanders has only been rigorously estimated in a few studies, our density and biomass estimates are higher than those of native salamanders associated with Appalachian streams (e.g., 2.68–3.57 individuals/m^2 for *D. monticola* [Bruce, 1995]; 1.13/m^2, 9.93 g/m^2 for *D. quadramaculatus* [Peterman et al., 2008]; 12/m^2, 7.88 g/m^2 for *D. quadrama-*

Table 3. Candidate closed capture model set and rankings analyzing the effect of age and time on capture (p) and recapture (c) probability of *D. monticola* at Spavinaw Creek. The best supported model is indicated in bold.

Model	QAICc	Δ QAICc*	w[†]	Model likelihood	K[‡]
p[a](age*time) = c[b](age*time)	**323.85**	**0.00**	**1.00**	**1.00**	**14**
p(age) = c(age)	253.70	70.15	0.00	0.00	4
p(time) = c(time)	245.74	78.11	0.00	0.00	7
p(.), c(.)	229.87	93.99	0.00	0.00	3
p(.) = c(.)	203.88	119.98	0.00	0.00	2

* Difference in QAICc relative to the top model
† QAICc weight
‡ Number of parameters in the model
[a] Capture and [b] Recapture probability, which vary by inclusion of the following covariates: age, time, or interactions of each, unless held constant (i.e., ".")

culatus, Gyrinophilus porphyriticus, and *Eurycea wilderae* [Davic, 1983; Peterman et al., 2008]; 1.85/m², 1.65g/m² for six species of riparian salamanders [Petranka and Murray, 2001]). In fact, based on manipulative experiments in streams, Kleeberger (1984) suggested that densities of *D. monticola* greater than ten individuals per m² are not possible naturally. It should also be noted that other sites along Spavinaw Creek likely host higher densities of *D. monticola* than our capture-mark-recapture site; during occupancy surveys, one site further upstream yielded nearly four times as many captures of *D. monticola* (Fig. 2). One reason for high densities of *D. monticola* may be a lack of natural controls on the introduced population. In its native range, *D. monticola* is frequently sympatric with other stream salamanders, including several congeners: *D. conanti, D. folkertsi, D. fuscus, D. marmoratus, D. ochrophaeus/orestes/ocoee, D. quadramaculatus,* and *D. welteri*. These species compete, but coexist through niche partitioning via differences in body size and microhabitat selection (Kryzysik, 1979; Hairston, 1986). Removal experiments have demonstrated that when other species of *Desmognathus* are excluded, the behavior and growth of *D. monticola* shifts to fill open niches (Southerland, 1986a, 1986b). In addition to lacking other species of *Desmognathus*, the Ozarks also lack other large stream salamander species such as *Gyrinophilus* and *Pseudotriton*, which are known to prey extensively on other salamanders (Petranka, 1998). Thus, streams in the Ozark region provide suitable habitat for *D. monticola* that is free from interspecific competition and intraguild predation.

Invasive species have the ability to suppress or displace native species through interference competition (Wu et al., 2005), predation (Davidson and Knapp, 2007), and numerous indirect effects. The high densities of *D. monticola* we documented and their potential role as a top predator in headwater streams suggest that they could alter Ozark stream food webs and threaten native species of salamanders and invertebrates. Of particular concern are two Ozark endemic salamanders that are found in Spavinaw Creek, *Eurycea spelaea* and *E. tynerensis*. *Desmognathus monticola* are known to be territorial and aggressive towards both conspecific and heterospecific salamanders (Keen and Sharp, 1984). Although frequency of predation by *Desmognathus* spp. on other salamanders can be low (Camp, 1997), *D. monticola* has been shown to reduce the abundance of other salamander species through predation (Hairston, 1986) and displacement (Keen, 1982). Paedomorphic populations of *E. tynerensis* occur in Spavinaw Creek, and this species is certainly small enough to be preyed upon by large *D. monticola*. Alternatively, larval *D. monticola* could negatively influence paedomorphic *E. tynerensis* or larval *E. spelaea* through exploitation

or interference competition within the aquatic habitat. Many Ozark streams are characterized by summer drying, which could concentrate salamanders into underground refugia and increase competitive or predatory interactions (Bonett et al., 2007). In our sampling, *D. monticola* were encountered much more frequently than native salamander species, but there was no clear pattern of displacement (Fig. 2). Thus, future research, such as comparisons of native salamander abundances in Spavinaw Creek over time or with nearby uninvaded tributaries, will be needed to fully understand the potential impacts of *D. monticola* on Ozark stream ecosystems.

This study also contributes to our understanding of pathways for introduction of non-native amphibians. *Desmognathus monticola* were likely introduced to Spavinaw Creek from Georgia by fishermen using salamanders as bait (Bonett et al., 2007). The first known location of *D. monticola* in Arkansas is at one of the few publicly accessible areas along Spavinaw Creek, which is a popular spot for anglers. Jensen and Waters (1999) reported that *D. monticola* was the most popular species of salamander ("spring lizard") being sold in Georgia bait shops, and Martof (1953) reported range expansions of *Desmognathus* salamanders in Georgia as a result of the "spring lizard" trade. Bait-buckets have proven to be a major induction pathway for non-native fish and crayfish (Kolar and Lodge, 2002; DiStefano et al., 2009), and it is likely that the bait trade is an important introduction vector for amphibians as well. In the western US, release of Tiger Salamander (*Ambystoma tigrinum*) larvae used as bait has led to the successful introduction of non-native forms, the hybridization between previously geographically separated species, and the spread of pathogens such as ranaviruses (Riley et al., 2003; Picco et al., 2007; Picco and Collins, 2008). Our documentation of successful invasion and spread of *D. monticola* in the Ozarks provides further evidence for the potential of the fishing bait trade as a vector for amphibian invasions.

We have demonstrated that *D. monticola* are firmly established over several kilometers of Spavinaw Creek and occur at high densities, but further research will be needed to assess their impacts and potential for continued spread. Once an alien species becomes firmly established, large-scale eradication is unlikely and even management or suppression is difficult and costly. It is likely that the population of *D. monticola* in Spavinaw Creek is already past the point of feasible eradication. Thus, understanding dispersal patterns and limitations will be needed to estimate potential expansion in the Ozarks. The data collected in this study form a baseline for tracking status and range of *D. monticola* over

time, and its relatively high species detection probability (0.4–0.8), will allow for rigorous monitoring of occupancy through low-intensity visual surveys. Continued monitoring and experimentation (e.g., removal experiments) will also be necessary to quantify the direct effects of invasion on native salamander and invertebrate populations, as well as potential indirect effects on trophic dynamics, nutrient cycling, and other ecosystem processes. Given the demonstrated track record of invasive herpetofauna to substantially alter native ecosystems (Kraus, 2015), these lines of research should be a high priority in Arkansas, as well as in surrounding states (Oklahoma, Missouri, Kansas) threatened by this novel invader.

ACKNOWLEDGMENTS

LITERATURE CITED

Abell, R., M. L. Thieme, C. Revenga, M. Bryer, M. Kottelat, N. Bogutskaya, B. Coad, N. Mandrak, S. C. Balderas, and W. Bussing. 2008. Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. BioScience 58:403–414.

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle, p. 267–281. *In*: Second International Symposium on Information Theory. B. N. Petrov and F. Cazaki (eds.). Akademiai Kiado, Budapest, Hungary.

Allan, J. D., and M. M. Castillo. 2007. Stream Ecology: Structure and Function of Running Waters. Springer, Netherlands.

Bonett, R. M., K. H. Kozak, D. R. Vieites, A. Bare, J. A. Wooten, and S. E. Trauth. 2007. The importance of comparative phylogeography in diagnosing introduced species: a lesson from the seal salamander, *Desmognathus monticola*. BioMed Central Ecology 7:7.

Bruce, R. C. 1995. The use of temporary removal sampling in a study of population dynamics of the salamander *Desmognathus monticola*. Australian Journal of Ecology 20:403–412.

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference. Springer-Verlag, New York.

Camp, C. D. 1997. The status of the black-bellied salamander (*Desmognathus quadramaculatus*) as a predator of heterospecific salamanders in Appalachian streams. Journal of Herpetology 31:613–616.

Casal, C. M. V. 2006. Global documentation of fish introductions: the growing crisis and recommendations for action. Biological Invasions 8:3–11.

Cecala, K. K., S. J. Price, and M. E. Dorcas. 2007. A comparison of the effectiveness of recommended doses of MS-222 (tricaine methanesulfonate) and Orajel® (benzocaine) for amphibian anesthesia. Herpetological Review 38:63–66.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18:117–143.

Clarke, K. R., and R. N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth.

Connette, G. M., and R. D. Semlitsch. 2012. Successful use of a passive integrated transponder (PIT) system for below-ground detection of plethodontid salamanders. Wildlife Research 39.1:1–6.

Connior, M. B., C. T. McAllister, H. W. Robison, and C. R. Bursey. 2013. Status of an exotic salamander, *Desmognathus monticola* (Caudata: Plethodontidae) and discovery of an introduced population of *Cottus immaculatus* (Perciformes: Cottidae) in Arkansas. Journal of the Arkansas Academy of Science 67:165–167.

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

Davic, R. D. 1983. An investigation of salamander guild predation in a North Carolina stream: an experimental approach. Unpubl. Ph.D. diss., Kent State University, Kent, Ohio.

Davidson, C., and R. A. Knapp. 2007. Multiple stressors and amphibian declines: dual impacts of pesticides and fish on Yellow-Legged Frogs. Ecological Applications 17:587–597.

DiStefano, R. J., M. E. Litvan, and P. T. Horner. 2009. The bait industry as a potential vector for alien crayfish introductions: problem recognition by fisheries agencies and a Missouri evaluation. Fisheries 34:586–597.

Dorazio, R. M., and J. A. Royle. 2005. Estimating size and composition of biological communities by modeling the occurrence of species. Journal of American Statistical Association 100:389–398.

Dorazio, R. M., J. A. Royle, B. Soderstrom, and A. Glimskar. 2006. Estimating species richness and accumulation by modeling species occurrence and detectability. Ecology 87:842–854.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A.-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163–182.

Evans, J. S., J. Oakleaf, S. A. Cushman, and D. Theobald. 2014. An ArcGIS Toolbox for Surface Gradient and Geomorphometric Modeling, version 2.0-0. Available: http://evansmurphy.wix.com/evansspatial. Accessed: 2 December 2014.

Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. Statistical Science 7:457–472.

Grant, E. H. C., J. D. Nichols, W. H. Lowe, and W. F. Fagan. 2010. Use of multiple dispersal pathways facilitates amphibian persistence in stream networks. Proceedings of the National Academy of Sciences of the United States of America 107:6936–6940.

Hairston, N. G. 1986. Species packing in *Desmognathus* salamanders: experimental demonstration of predation and competition. American Naturalist 127:266–291.

Hunt, S. D., J. C. Guzy, S. J. Price, B. J. Halstead, E. A. Eskew, and M. E. Dorcas. 2013. Responses of riparian reptile communities to damming and urbanization. Biological Conservation 157:277–284.

Hyde, E. J., and T. R. Simons. 2001. Sampling plethodontid salamanders: sources of variability. Journal of Wildlife Management 65:624–632.

Iverson, L. R., M. E. Dale, C. T. Scott, and A. Prasad. 1997. A GIS derived integrated moisture index to predict forest composition and productivity of Ohio forests (U.S.A.). Landscape Ecology 12:331–348.

Jensen, J., and C. Waters. 1999. The Spring Lizard bait industry in the state of Georgia, USA. Herpetological Review 30:20–21.

Keen, W. H. 1982. Habitat selection and interspecific competition in two species of plethodontid Salamanders. Ecology 63:94–102.

Keen, W. H., and S. Sharp. 1984. Responses of a plethodontid salamander to conspecific and congeneric intruders. Animal Behavavior 32:58–65.

Kéry, M., J. A. Royle, M. Plattner, and R. M. M. Dorazio. 2009. Species richness and occupancy estimation in communities subject to temporary emigration. Ecology 90:1279–1290.

Kleeberger, S. R. 1984. A test of competition in two sympatric populations of desmognathine salamanders. Ecology 65:1846–1856.

Kolar, C. S., and D. M. Lodge. 2002. Ecological predictions and risk assessment for alien fishes in North America. Science 298:1233–1236.

Kraus, F. 2015. Impacts from invasive reptiles and amphibians. Annual Review of Ecology, Evolution and Systematics 46:75–97.

Krzysik, A. J. 1979. Resource allocation, coexistence, and the niche structure of a streambank salamander community. Ecological Monograph 49:173–194.

Link, W. A., E. Cam, J. D. Nichols, and E. G. Cooch. 2002. Of BUGS and birds: Markov Chain Monte Carlo for hierarchical modeling in wildlife research. Journal of Wildlife Management 66:277–291.

Lowe, S., M. Browne, S. Boudjelas, and M. De Poorter. 2004. 100 of the World's Worst Invasive Alien Species: A Selection From the Global Invasive Species Database. The Invasive Species Specialist Group, Species Survival Commission, World Conservation Union.

Lowe, W. H., G. E. Likens, M. A. McPeek, and D. Buso. 2006. Linking direct and indirect data on dispersal: isolation by slope in a headwater stream salamander. Ecology 87:334–339.

Lunn, D. J., A. Thomas, N. Best, and D. Spiegelhalter. 2000. WinBUGS—a Bayesian modelling framework: concepts, structure, and extensibility. Statistical Computing 10:325–337.

Marold, M. R. 2001. Evaluating visual implant elastomer polymer for marking small, stream-dwelling salamanders. Herpetological Review 32:91–92.

Martof, B. 1953. The "Spring Lizard" industry: a factor in salamander distribution and genetics. Ecology 34:436–437.

Meyer, J., L. Kaplan, J. Newbold, D. Strayer, C. Woltemade, J. Zelder, R. Beilfuss, Q. Carpenter, R. Semlitsch, and M. Watzin. 2003. Where Rivers are Born: The Scientific Imperative for Defending Small Streams and Wetlands. American Rivers and Sierra Club.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman, and N. E. Leonard. 2007. The contribution of headwater streams to biodiversity in river networks. Journal of American Water Resources Association 43:86–103.

Mooney, H. A., and E. E. Cleland. 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences of the United States of America 98:5446–5451.

Moyle, P. B., and M. P. Marchetti. 2006. Predicting invasion success: freshwater fishes in California as a model. BioScience 56:515–524.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs 62:7–135.

Peterman, W. E., J. A. Crawford, and R. D. Semlitsch. 2008. Productivity and significance of headwater streams: population structure and biomass of the black-bellied salamander (Desmognathus quadramaculatus). Freshwater Biology 53:347–357.

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

Petranka, J. W., and S. S. Murray. 2001. Effectiveness of removal sampling for determining salamander density and biomass: a case study in an Appalachian streamside community. Journal of Herpetology 35:36–44.

Picco, A. M., J. L. Brunner, and J. P. Collins. 2007. Susceptibility of the endangered California tiger salamander, Ambystoma californiense, to ranavirus infection. Journal of Wildlife Diseases 43:286–290.

Picco, A. M., and J. P. Collins. 2008. Amphibian commerce as a likely source of pathogen pollution. Conservation Biology 22:1582–1589.

Pitt, W., D. Vice, and W. Pitzler. 2005. Challenges of Invasive Reptiles and Amphibians. Wildlife Damage Management Conferences—Proceedings. Paper 84.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Riley, S. P., B. H. Shaffer, V. S. Randal, and B. M. Fitzpatrick. 2003. Hybridization between a rare, native tiger salamander (Ambystoma californiense) and its introduced congener. Ecological Applications 13:1263–1275.

Rupp, H. R. 1996. Adverse assessments of Gambusia affinis: an alternative view for mosquito control practitioners. Journal of American Mosquito Control 12:155–166.

Sauer, J. R., and W. A. Link. 2002. Hierarchical modeling of population stability and species group attributes from survey data. Ecology 86:1743–1751.

Shireman, J. V. 1984. Control of aquatic weeds with exotic fishes, p. 302–312. In: Distribution, Biology, and Management of Exotic Fishes. W. R. Courtenay, Jr. and J. R. Stauffer, Jr. (eds.). The John Hopkins University Press, Baltimore, Maryland.

Simon, K. S., and C. R. Townsend. 2003. Impacts of freshwater invaders at different levels of ecological organisation, with emphasis on salmonids and ecosystem consequences. Freshwater Biology 48:982–994.

Smith, R. K., P. L. Freeman, J. V. Higgins, K. S. Wheaton, T. W. FitzHugh, K. J. Ernstrom, and A. A. Das. 2002. Priority Areas for Freshwater Conservation Action: A Biodiversity Assessment of the Southeastern United States. The Nature Conservancy.

Snodgrass, J. W., D. C. Forester, M. Lahti, and E. Lehman. 2007. Dusky salamander (Desmognathus fuscus) nest-site selection over multiple spatial scales. Herpetologica 63:441–449.

Southerland, M. T. 1986a. Behavioral interactions among four species of the salamander genus Desmognathus. Ecology 67:175–181.

Southerland, M. T. 1986b. Coexistence of three congeneric salamanders: the importance of habitat and body size. Ecology 67:721–728.

Sturtz, S., U. Ligges, and A. Gelman. 2005. R2WinBUGS: a package for running WinBUGS from R. Journal of Statistical Software 12:1–16.

TNC (The Nature Conservancy, Ozarks Ecoregional Assessment Team). 2003. Ozarks Ecoregional Conservation Assessment. The Nature Conservancy Midwestern Resource Office, Minneapolis, Minnesota.

Trauth, S. E., H. W. Robison, and M. V. Plummer. 2004. The Amphibians and Reptiles of Arkansas. University of Arkansas, Fayetteville, Arkansas.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. Journal of Geology 30:377–392.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46:S120–S139.

Wu, Z. J., Y. M. Li, Y. P. Wang, and M. J. Adams. 2005. Diet of introduced bullfrogs *Rana catesbeaina*: predation on and diet overlap with native frogs on Daishan Island, China. Journal of Herpetology 39:668–674.

Zipkin, E. F., A. Dewan, and J. A. Royle. 2009. Impacts of forest fragmentation on species richness: a hierarchical approach to community modeling. Journal of Applied Ecology 46:815–822.