

## Factors Influencing the Occupancy and Abundance of Streamside Salamander (*Ambystoma barbouri*) in Kentucky Streams

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**ABSTRACT.**—For many amphibian species of conservation concern, our understanding of drivers of distribution and abundance are based on data collected at only a few localities. Thus, landscape-scale efforts are needed to better inform management and species conservation. The Streamside Salamander (*Ambystoma barbouri*) is a global conservation priority because of its restricted geographic distribution and presumed sensitivity to forest loss, yet landscape-scale studies have not occurred. We surveyed a 16-county area in Kentucky (USA) and examined the influence of catchment (forest, agriculture, and urban) and local-scale (water chemistry and sunfish presence) variables on *A. barbouri* occupancy and abundance. We sampled 80 streams across different land cover types (i.e., 30 agriculture, 34 forested, and 16 urban) over 2 yrs (2017–2018). Within these streams, we identified 56 new localities. Estimated mean occupancy ( $\Psi = 0.91$ ; credible interval [CI] = 0.76–0.99) was high. Streams with the highest estimated abundances were those without sunfish and containing >50% forested or agricultural land cover within their catchments; urban streams had the lowest estimated abundances. At sites where we did not detect sunfish, there were an estimated 35.25 (95% CI = 32.04–38.67) *A. barbouri* individuals per 10 m in forested sites, 22.47 (95% CI = 19.86–25.14) in agricultural sites, and 0.66 (95% CI = 0.14–1.59) in urban sites. Water chemistry varied across site types; however, only maximum sodium ( $\beta = -0.51$ , 95% CI = -0.58 to -0.43) was associated with *A. barbouri* abundance. If managing this species becomes necessary, minimizing urban development within low-order stream catchments should be a priority.

Understanding factors influencing species distribution and abundance are important for defining priorities for amphibian conservation and management. However, for many species of conservation concern, understanding drivers of distribution and abundance is based on expert opinion, presence-only data, or data collected at only a few localities (Loiselle et al., 2003; Johnson and Gillingham, 2004; Dillard et al., 2008). Although these data sources often represent the best-available information, they potentially introduce bias into species assessment and identification of critical habitats (Bodinof Jachowski et al., 2016). Thus, for many species it remains unclear whether inferences gained from these limited assessments are useful for conservation and management action.

The Streamside Salamander (*Ambystoma barbouri*; Kraus and Petranka, 1989) is a stream-breeding mole salamander (Ambystomatidae) with a spotty distribution primarily within north-central Kentucky and southern Ohio and Indiana, with disjunct populations in Tennessee, West Virginia, and western Kentucky (USA). *Ambystoma barbouri* is considered a conservation priority in Ohio, Indiana, West Virginia, and Tennessee and is listed as “near threatened” by the International Union for Conservation of Nature (IUCN, 2018). Only in Kentucky, which constitutes >50% of its range, is *A. barbouri* considered stable (Kentucky Department of Fish and Wildlife Resources, 2013). Previous studies indicate that larvae decline following flood events (Petranka and Sih, 1986) and are sensitive to fish (Centrarchidae) predation (Sih et al., 1992; Sih and Kats, 1994) and agricultural chemicals (Rehage et al., 2002; Rohr et al., 2003, 2004, 2006, 2011; Rohr and Palmer, 2005). Adults are highly fossorial and presumed to be strongly associated with forested landscapes (Petranka, 1998). *Ambystoma barbouri* is assumed to be declining throughout its range due to habitat destruction, specifically the degradation of low-order streams and conversion of forested land to agricultural land and urban development (see Petranka, 1998; Niemiller et al., 2006; Kraus, 2013).

Yet, the vast majority of previous research has been conducted only at a few localities in central Kentucky and Tennessee (e.g., Petranka, 1998; Niemiller et al., 2006), and it remains unclear whether inferences gained from these studies extend throughout a broader portion of the *A. barbouri* range.

We surveyed gaps in the geographic distribution of *A. barbouri* in a 16-county area of northeastern and central Kentucky (Fig. 1) and examined the relationships between occupancy and abundance with local and catchment-scale habitat conditions. Specifically, we examined relationships between *A. barbouri* occupancy and abundance and water chemistry, sunfish presence, and land cover within stream catchments. Our findings provide an assessment of the current status of *A. barbouri* throughout a broader portion of its geographic range and indicate potential threats and management options for this regionally endemic species.

### MATERIALS AND METHODS

**Study Sites.**—We focused sampling efforts on low-order streams in northeastern and central Kentucky counties where limited *A. barbouri* occurrence data exist. Using a geographic information system (ArcMap 10.4.1, ESRI), we focused efforts on first- and second-order streams within U.S. Geological Survey 7.5-min quadrangles without occurrence data ( $N = 41$ ) in the targeted region. We randomly selected half of these quadrangles for each year of sampling. We used Hydrology Tools within ArcMap to delineate catchments and calculate drainage area. We defined catchments as the area of the drainage basin upstream of each sampling reach; catchments ranged from 19.81 to 1,058.40 ha (mean = 262.84 ha). We used National Land Cover Data (Fry et al., 2011) to obtain dominant land cover within each stream’s catchment. We grouped land-use categories as agriculture (herbaceous, hay/pasture, and cultivated crops), forest (deciduous, evergreen, mixed, and shrub/scrub), and urban (developed open space, developed low/medium/high intensity, and barren land), and we calculated percentages of each category within

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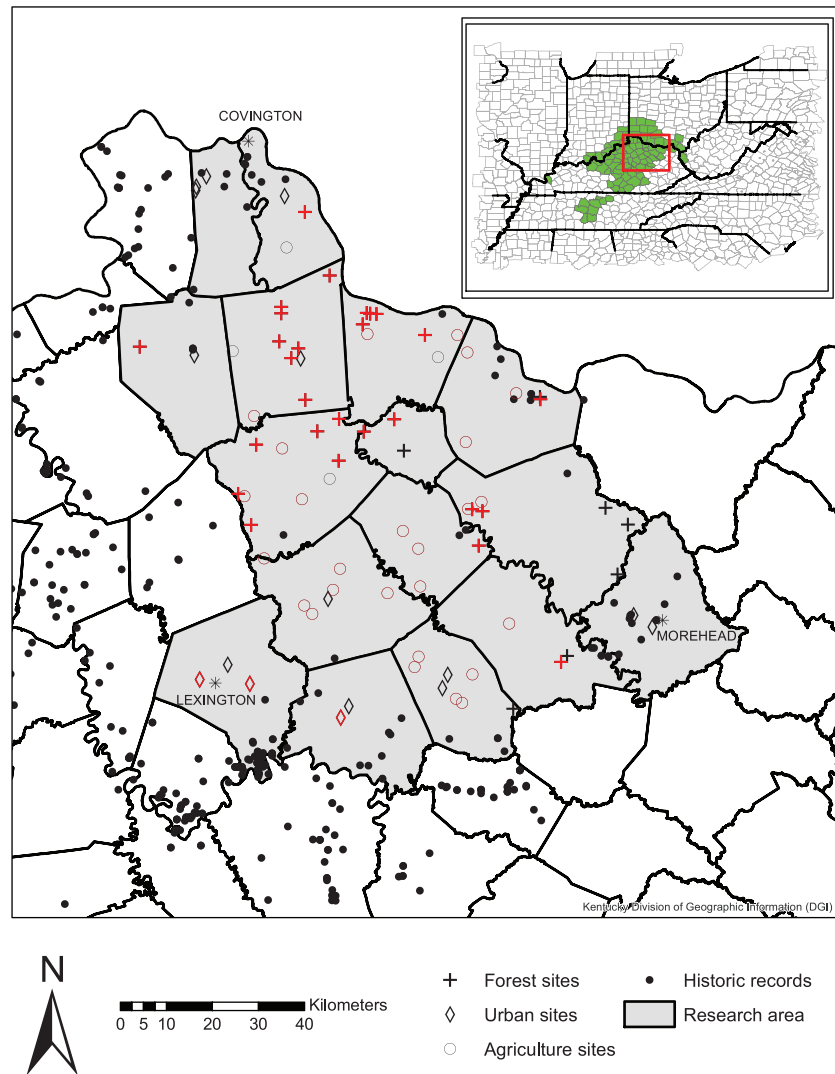


FIG. 1. Location of *Ambystoma barbouri* study streams in northeastern and central Kentucky, delineated by site type (agriculture [ $N = 30$ ], forested [ $N = 34$ ], and urban [ $N = 16$ ]). Sites in red indicate new *A. barbouri* localities.

each stream catchment. Stream categories were defined based on the dominant land-use type within each catchment, as determined by >50% of total land use for agriculture and forest categories and >33% for urban sites. We used >33% to delineate urban streams because impervious surface, a main component of urban land use, can dramatically decrease biological diversity within streams even for watersheds containing values as low as 8–33% (Paul and Meyer, 2001). Ultimately, we sampled 80 streams (30 agriculture, 34 forested, and 16 urban catchments) for *A. barbouri* across a 16-county area (Fig. 1). Sampling was conducted over 2 yr and occurred from 29 March to 1 June 2017 ( $N = 40$ ) and from 9 April to 4 June 2018 ( $N = 40$ ).

**Amphibian Sampling.**—We used area-constrained active searches to sample *A. barbouri* at 10-m reaches for each stream. We chose stream reaches with at least one deep (i.e., 30–60-cm) pool (Watson and Pauley, 2005; Yeiser and Richter, 2015). We used systematic dipnetting and cover object searches to detect larval *A. barbouri* (Price et al., 2012). Dipnetting consisted of one person, moving upstream, actively dipnetting for *A. barbouri* among submerged cover objects within the stream and pools for 30 min. Larvae were temporarily removed until the search was complete and then counted. Each transect was sampled on three occasions

during daylight hours with at least 10 days between samples. We recorded water temperature and sunfish presence (i.e., known predators of *A. barbouri*; Petranks, 1983; Sih et al., 1992; Sih and Kats, 1994) at each active search. Sunfish were exclusively recorded due to their voracious appetite for *A. barbouri* (28 larvae/15 min) compared with that of other fish species (1–4 larvae/15 min; Petranks, 1983).

**Water Chemistry.**—We collected water samples to characterize differences among land-use categories. Before each active search, two 250-mL water samples were collected and placed on ice. All water samples were analyzed for concentrations of total organic carbon, pH, alkalinity, chloride, sulfate, nitrate, ammonium, calcium, magnesium, potassium, sodium, nitrite, iron, manganese, total suspended solids, and specific conductance. Water quality sampling, preservation, and analytic protocols were performed in accordance with standard methods (Greenberg et al., 1992). Previous research suggests sensitivity of ambystomids to elevated nitrate, sodium, and conductivity (i.e., Chambers, 2011; Egea-Serrano et al., 2012). Because conductivity can be influenced by multiple inputs, and sodium is positively correlated to and can influence conductivity readings (Bhandari and Nayal, 2008), we focused solely on nitrate and sodium for

our occupancy and abundance analyses. Water quality analyses were conducted at the Forest Hydrology Lab at the University of Kentucky.

Water chemistry data were analyzed using a repeated measures analysis of variance (ANOVA) in SPSS 24 (IBM Corp.) to assess the effects of land use (forest, agriculture, and urban) on water quality parameters. Data were checked for sphericity (Mauchly, 1940). For parameters in which sphericity could not be assumed, we used the Greenhouse–Geisser correction for  $\epsilon < 0.75$  and the Huynh–Feldt correction for  $\epsilon > 0.75$  ( $\epsilon$  Greenhouse and Geisser, 1959; Huynh and Feldt, 1976). These corrections function by decreasing degrees of freedom, thereby correcting for violations in the sphericity assumption. Significant differences detected by ANOVA were further analyzed using Tukey's pairwise comparison test (Tukey, 1949).

**Occupancy Analyses.**—We used a single-season Bayesian occupancy model (Kéry and Schaub, 2012) to estimate larval *A. barbouri* response to site-specific covariates (land use category, water chemistry composition, and sunfish presence) and sampling covariates expected to influence detection probability (day of year and water temperature). We generated an observance matrix for three sampling occasions at each site. Thus, the data were structured as a matrix  $x(i,j)$  for occupancy of site  $i$  for the  $j$ th sampling occasion. We related covariate parameters ( $\alpha$  and  $\beta$  values, described below) and occupancy and detection probabilities ( $\Psi_i$  and  $\Theta_{ij}$ , respectively) with the model below (effects parameterization):

$$\text{logit}(\Psi_i)\alpha_0 + \alpha_1\text{urban}_i + \alpha_2\text{agriculture}_i + \alpha_3\text{fish}_i + \alpha_4\text{nitrate}_i + \alpha_5\text{sodium}_i$$

We modeled detection probabilities for each species with the following equation, within the model described above:

$$\text{logit}(\Theta_{ij}) = \beta_0 + \beta_1\text{day of year}_{1j} + \beta_2\text{water temperature}_j$$

Parameters  $\alpha_1$  and  $\alpha_2$  were effects of the categorical predictor variable “Dominant Land Cover,” with “Forested” as the reference category, and parameter  $\alpha_3$  was the effect of the categorical predictor variable “Sunfish Presence,” with “Sunfish Absent” as the reference category. Continuous covariates (i.e., nitrate, sodium, day of year, and water temperature) were scaled such that the mean value was zero (i.e., site's nitrate value – mean/SD).

The model contained nine parameters:  $\alpha_{0i}$ ,  $\alpha_{1i}$ ,  $\alpha_{2i}$ ,  $\alpha_{3i}$ ,  $\alpha_{4i}$ ,  $\alpha_{5i}$ ,  $\beta_{0i}$ ,  $\beta_{1i}$ , and  $\beta_{2i}$ . Standardized covariates allowed us to estimate  $\Psi$  and  $\Theta$  at mean site and survey covariates (where the mean = 0) and allowed direct comparison of model coefficients as effect sizes relative to variation in each covariate. We organized all data in R 3.3.2 (R Development Core Team, 2015) and executed analyses in WinBUGS (Spiegelhalter et al., 2003) using R2WinBUGS (Sturtz et al., 2005). This model, and abundance models (see below; Abundance Analyses), were implemented in a Bayesian framework using Markov chain Monte Carlo sampling in WinBUGS to generate samples from the posterior distribution (Lunn et al., 2013).

We used uninformative priors for all parameters (i.e., U (0, 0.01)). For this model, and abundance models (see below; Abundance Analyses), three parallel chains were run in WinBUGS for each model so that convergence could be assessed via the Gelman–Rubin diagnostic. For all monitored parameters in the study, this value was at or below 1.02 (Gelman and Rubin, 1992). For this model, and abundance models (see below; Abundance Analyses), each chain was run for 200,000 iterations in total, the first 100,000 were removed as burn-in, and the

remainder were thinned by a factor of 50. Across the three chains, this provided 6,000 samples for both the occupancy and abundance models to approximate posterior summary statistics for each model parameter including mean, SD, and 2.5 and 95% percentiles of the distribution, which represent 95% Bayesian credible intervals (CIs). CIs are defined by quantiles of the posterior distribution. For this model, and abundance models (see below; Abundance Analyses), we inferred support for continuous covariates when intervals did not contain zero. Occupancy and detection estimates were derived using the inverse logit transformation (i.e.,  $(\exp(\alpha))/(1 + \exp(\alpha))$ ).

**Abundance Analyses.**—We used a binomial mixture model (Royle, 2004) to examine effects of site-specific covariates (land-use category, water chemistry composition, and sunfish presence) and sampling covariates (day of year and water temperature) expected to influence abundance estimates of larval *A. barbouri*. We conducted three replicate count surveys at 80 spatially distinct sites ( $i$ ) during temporally indexed surveys ( $j$ ), denoted as  $c_{ij}$  (Royle and Dorazio, 2008). Under this framework, counts were modeled as independent outcomes of binomial sampling with index  $N_i$  and detection probability  $p_j$ . Abundances ( $\lambda$ ) at the local level were modeled with a Poisson distribution. Heterogeneity in abundance among populations due to site-specific covariates ( $x_i$ ) was modeled using a Poisson-regression formulation of local mean abundances, given by  $\log(\lambda_i) = \beta_0 + \beta_1 x_i$ . Sources of heterogeneity in detection were identified by modeling associations between sampling covariates and  $p_j$  such that  $\text{logit}(p_{ij}) = \alpha_0 + \alpha_1 x_{ij}$ . See Price et al. (2013) for further model description.

We organized count data by site and survey and specified larval *A. barbouri* abundance with the model below. Parameters  $\alpha_1$  and  $\alpha_2$  were effects of the categorical predictor variable “Dominant Land Cover,” with “Forested” as the reference category, and parameter  $\alpha_5$  was the effect of the categorical predictor variable “Sunfish Presence,” with “Sunfish Absent” as the reference category. Continuous covariates (i.e., nitrate, sodium, day of year, and water temperature) were scaled such that the mean value was zero (i.e., site's nitrate value – mean/SD).

$$N_i | \lambda_i \sim \text{Poi}(\lambda_i)$$

$$\log(\lambda_i) = \beta_0 + \beta_1\text{urban} + \beta_2\text{agriculture} + \beta_3\text{nitrate} + \beta_4\text{sodium} + \beta_5\text{fish}$$

Heterogeneity in detection probability was modeled with the following equation included within the model described above:

$$c_{ij} | N_i \sim \text{Bin}(N_i, p_{ij})$$

$$\text{logit}(p_{ij}) = \alpha_0 + \alpha_1\text{day of year} + \alpha_2\text{water temperature}$$

Models used uninformative priors; specifically, we assumed  $\beta_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5 \sim N(0, 10^2)$ ,  $\alpha_0 \sim N(0, 1.6^2)$ , and  $\alpha_1, \alpha_2 \sim N(0, 10^2)$ . The  $\alpha_0$  prior approximates a U (0, 1) prior for  $\exp(\alpha_0)$ , where  $\exp$  represents the inverse logit function (i.e.,  $\exp(\alpha)/(1 + \exp(\alpha))$ ).

## RESULTS

**Distribution.**—We identified 56 new locations for *A. barbouri* within our 80 sampled stream reaches. Over all sampling periods within sampled stream reaches, we captured 2,713 *A. barbouri* larvae (forested sites  $N = 34$ , larvae  $N = 1,859$ ; agriculture sites  $N = 30$ , larvae  $N = 850$ ; urban sites  $N = 16$ , larvae  $N = 4$ ). In addition,

TABLE 1. Water chemistry attributes for streams in northeastern and central Kentucky. Estimates reported include the mean  $\pm$  SE for water chemistry across site types, including specific conductance (Cond), total organic carbon (TOC), pH, alkalinity, chloride (Cl), sulfate (SO<sub>4</sub>), nitrate (NO<sub>3</sub>-N), ammonium (NH<sub>4</sub>-N), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), nitrite (NO<sub>2</sub>-N), iron (Fe), manganese (Mn), and total suspended solids (TSS). Bold type and lowercase superscript letters indicate significant differences among treatments; site types that do not share a letter are significantly different.

Water quality parameter	Agriculture	Forest	Urban
Cond	<b>428.64 <math>\pm</math> 8.16<sup>a</sup></b>	<b>429.55 <math>\pm</math> 15.44<sup>a</sup></b>	<b>584.60 <math>\pm</math> 29.18<sup>b</sup></b>
TOC	8.76 $\pm$ 0.67 <sup>a</sup>	8.13 $\pm$ 0.67 <sup>a</sup>	8.04 $\pm$ 0.79 <sup>a</sup>
PO <sub>4</sub>	6.60 $\pm$ 1.46 <sup>a</sup>	8.68 $\pm$ 2.06 <sup>a</sup>	10.49 $\pm$ 3.69 <sup>a</sup>
pH	7.4 $\pm$ 0.03 <sup>a</sup>	7.85 $\pm$ 0.62 <sup>a</sup>	7.29 $\pm$ 0.05 <sup>a</sup>
Alkalinity	280.35 $\pm$ 4.02 <sup>a</sup>	282.74 $\pm$ 22.72 <sup>a</sup>	253.44 $\pm$ 9.63 <sup>a</sup>
Cl	9.72 $\pm$ 2.04 <sup>a</sup>	9.54 $\pm$ 1.50 <sup>a</sup>	11.30 $\pm$ 2.39 <sup>a</sup>
SO <sub>4</sub>	49.24 $\pm$ 3.18 <sup>a</sup>	59.95 $\pm$ 6.67 <sup>a</sup>	44.16 $\pm$ 5.30 <sup>a</sup>
NO <sub>3</sub> -N	<b>0.77 <math>\pm</math> 0.10<sup>a</sup></b>	<b>0.09 <math>\pm</math> 0.03<sup>b</sup></b>	<b>0.65 <math>\pm</math> 0.09<sup>a</sup></b>
NH <sub>4</sub> -N	0.08 $\pm$ 0.01 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>a</sup>
Ca	40.56 $\pm$ 0.74 <sup>a</sup>	36.99 $\pm$ 1.40 <sup>a</sup>	42.38 $\pm$ 1.29 <sup>a</sup>
Mg	7.66 $\pm$ 0.22 <sup>a</sup>	8.35 $\pm$ 0.21 <sup>a</sup>	7.63 $\pm$ 0.26 <sup>a</sup>
K	2.36 $\pm$ 0.10 <sup>a</sup>	2.40 $\pm$ 0.07 <sup>a</sup>	2.27 $\pm$ 0.13 <sup>a</sup>
Na	<b>6.94 <math>\pm</math> 0.31<sup>a</sup></b>	<b>7.47 <math>\pm</math> 0.32<sup>a</sup></b>	<b>14.85 <math>\pm</math> 0.34<sup>b</sup></b>
NO <sub>2</sub> -N	0.19 $\pm$ 0.03 <sup>a</sup>	0.12 $\pm$ 0.01 <sup>a</sup>	0.13 $\pm$ 0.02 <sup>a</sup>
Fe	0.01 $\pm$ 0.00 <sup>a</sup>	0.01 $\pm$ 0.00 <sup>a</sup>	0.00 $\pm$ 0.00 <sup>a</sup>
Mn	0.01 $\pm$ 0.01 <sup>a</sup>	0.01 $\pm$ 0.00 <sup>a</sup>	0.01 $\pm$ 0.00 <sup>a</sup>
TSS	12.87 $\pm$ 1.19 <sup>a</sup>	16.37 $\pm$ 1.86 <sup>a</sup>	8.04 $\pm$ 2.08 <sup>a</sup>

we observed *A. barbouri* in 14 of 16 sampled counties (not detected in Kenton and Rowan).

**Water Chemistry.**—Water quality parameters varied significantly among land-use categories (Wilk's Lambda = 0.11,  $F_{34,84} = 5.12$ ,  $P < 0.001$ ). Of 17 water quality parameters, 3 varied significantly among land use types including sodium ( $F_{2,58} = 37.49$ ,  $P < 0.001$ ), conductivity ( $F_{2,58} = 7.72$ ,  $P = 0.001$ ), and nitrate ( $F_{2,58} = 11.18$ ,  $P <$

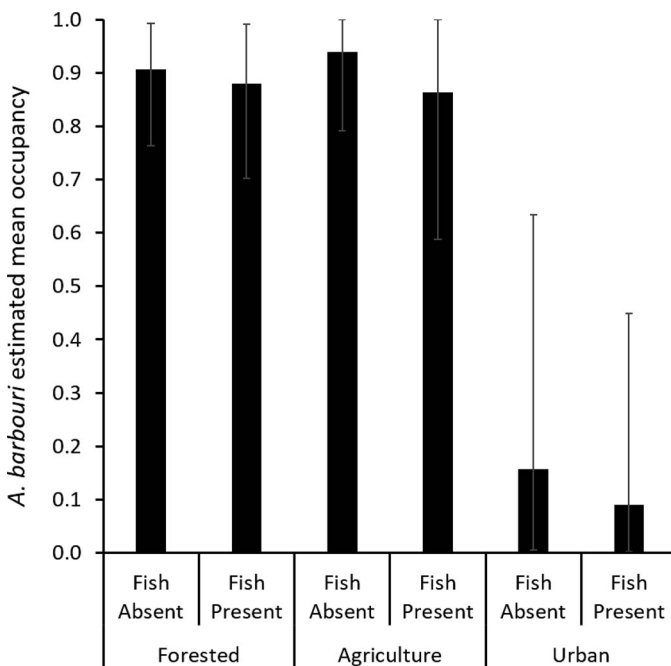


FIG. 2. Estimated mean occupancy of larval *Ambystoma barbouri* by site type (forested, agriculture, and urban) and sunfish presence/absence for streams in northeastern and central Kentucky. Error bars indicate 95% CI.

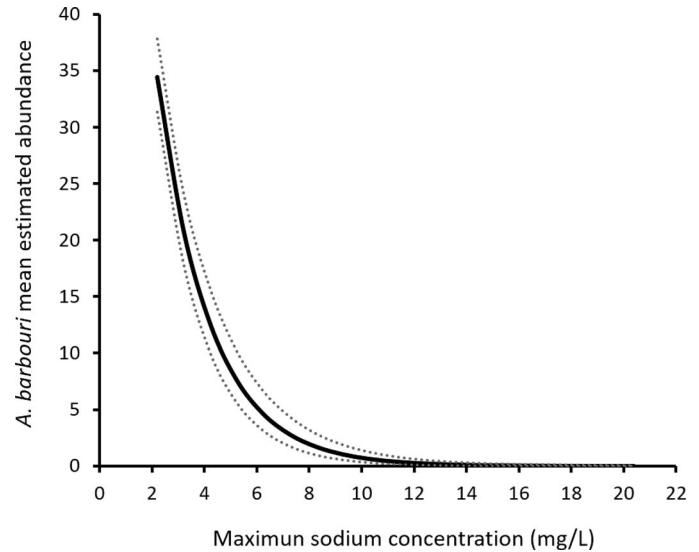


FIG. 3. Estimated mean abundance of larval *Ambystoma barbouri* by maximum sodium concentration (milligrams per liter) for streams in northeastern and central Kentucky. Dotted lines indicate 95% CI.

0.001). Water collected from urban sites had the highest sodium concentrations, conductivity, and along with agriculture sites, the highest nitrate (Table 1). The mean sodium concentration in the collected water was nearly double for urban sites ( $14.85 \pm 0.34$  mg/L) compared with agriculture ( $6.94 \pm 0.31$  mg/L) and forested ( $7.47 \pm 0.32$  mg/L) sites. In addition, conductivity was slightly higher for urban sites ( $584.60 \pm 29.18$ ) than forested ( $429.55 \pm 15.44$ ) and agriculture ( $428.64 \pm 8.16$ ) sites. Nitrate concentrations, although low overall, were higher in both agriculture ( $0.77 \pm 0.10$  mg/L) and urban ( $0.65 \pm 0.09$  mg/L) sites than in forested sites ( $0.09 \pm 0.03$  mg/L).

**Occupancy Estimates.**—Estimated mean occupancy across all site was 0.91 (95% CI = 0.76–0.99). The estimated occupancy of *A. barbouri* was positively associated with forested sites ( $\alpha_0 = 2.56$ , 95% CI = 1.17–4.89), and we did not detect a difference between forested sites and agricultural sites ( $\alpha_2 = 1.671$ , 95% CI = –1.706 to 14.3). Conversely, estimated *A. barbouri* occupancy of urban sites was lower than that of forested sites ( $\alpha_1 = -4.78$ , 95% CI = –9.00 to –1.75). Fish presence did not influence occupancy estimates ( $\alpha_3 = -0.85$ , 95% CI = –2.80 to 1.36; Fig. 2). Mean estimated detection probability across all site types was 0.73 (95% CI = 0.64–0.81), and detection probability was not influenced by day of year ( $\beta_1 = -0.11$ , 95% CI = –0.47 to 0.25) or water temperature ( $\beta_2 = -0.21$ , 95% CI = –0.59 to 0.16).

**Abundance Estimates.**—Estimated abundance of larval *A. barbouri* was positively associated with forested sites ( $\beta_0 = 3.56$ , 95% CI = 3.47–3.66), and abundance was lower at agriculture ( $\beta_2 = -0.45$ , 95% CI = –0.59 to –0.32) and urban ( $\beta_1 = -4.15$ , 95% CI = –5.53 to –3.08) sites than at forested sites. Similarly, sites containing sunfish had fewer estimated *A. barbouri* than those without sunfish ( $\beta_5 = -1.01$ , 95% CI = –1.20 to –0.83). Estimated abundance was negatively associated with maximum sodium concentration ( $\beta_4 = -0.51$ , 95% CI = –0.58 to –0.43; Fig. 3). Nitrate concentration did not influence estimated abundance ( $\beta_3 = -0.02$ , 95% CI = –0.08 to 0.04).

At sites where we did not detect fish, we estimated an average of 35.25 (95% CI = 32.04–38.67) larval *A. barbouri* individuals per 10 m reach in forested sites, 22.47 (95% CI = 19.86–25.14) in agricultural sites, and 0.66 (95% CI = 0.14–1.59)

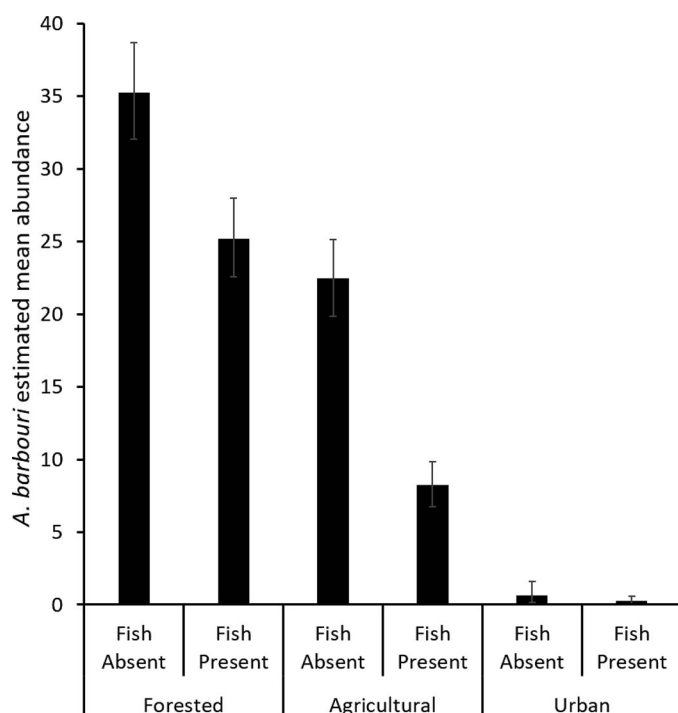


FIG. 4. Estimated mean abundance of larval *Ambystoma barbouri* by site type (forested, agriculture, and developed/urban watersheds) and sunfish presence/absence for streams in northeastern and central Kentucky. Error bars indicate 95% CI.

in urban sites (Fig. 4). Forested sites containing sunfish were estimated to have an average of 25.19 (95% CI = 22.56–28.00) larval *A. barbouri* individuals per 10 m reach compared with 8.22 at agriculture (95% CI = 6.73–9.83) and 0.24 at urban (95% CI = 0.05–0.59) sites. Day of year ( $\alpha_1 = 0.04$ , 95% CI = -0.01 to 0.10) and water temperature ( $\alpha_2 = -0.01$ , 95% CI = -0.07 to 0.05) did not influence per individual detection probability. Mean per-individual detection probability was estimated to be 0.43 (95% CI = 0.40–0.45).

#### DISCUSSION

Our landscape-scale investigation of *A. barbouri* filled the species distribution gap in the central portion of its range, documenting the species at several new locations. Estimated mean occupancy and detection estimates across the study area were high at 0.91 and 0.73, respectively, indicating that this species is common in northeastern and central Kentucky and that detection of this species is likely if it is present in a stream. Our abundance estimates among site types are generally consistent with previous research on preferred habitat conditions (Kraus and Petranka, 1989; Petranka, 1998). Namely, streams with mostly (i.e., >50%) forested catchments without sunfish had the highest larval *A. barbouri* abundances; however, we also documented high *A. barbouri* occupancy and abundance in catchments dominated by agriculture without sunfish, contrary to previous knowledge on this species. As expected, streams located in urban areas had the fewest *A. barbouri*. Water chemistry varied across site types, but only sodium was negatively correlated with *A. barbouri* abundance.

*Ambystoma barbouri* is generally thought to be associated with low-order, fishless streams within gently sloping upland deciduous forests (Kraus and Petranka, 1989). Yeiser and Richter (2005) sampled three streams in forested catchments

and found larval densities as high as 90 individuals/m<sup>2</sup>. Our landscape-scale investigation found *A. barbouri* abundances were greatest in streams that match these conditions. However, we also found high *A. barbouri* occupancy and abundance in agriculture catchments without sunfish. Kraus (2013) suggests *A. barbouri* can persist despite forest fragmentation via the use of small remnant forests within these landscapes, but they are unable to occupy areas that are cleared of all forests. Our research suggests that *A. barbouri* may not be as tied to forested habitat as previously thought and can occupy and reach high larval abundances in streams without sunfish in agricultural areas with no forest cover. Urban sites had the fewest larval *A. barbouri* in our study area likely because urban environments present a suite of potential impediments to salamanders (Barrett and Price, 2014) via the complex web of stressors known as the “urban stream syndrome” (Walsh et al., 2005). For example, *A. barbouri* adults, eggs, and larvae may be susceptible to high peak flow events (Petranka and Sih, 1986), road mortality during adult and juvenile migrations (Andrews et al., 2008), multiple chemical stressors (e.g., motor oil, heavy metals, nutrients; Egea-Serrano et al., 2012), and limited availability of upland, forested habitat (Cushman, 2006).

Consistent with previous research conducted at smaller scales, our research indicates streams without sunfish predators have greater *A. barbouri* abundance. Previous laboratory research has documented heavy sunfish predation on larvae of *A. barbouri* (i.e., 28 larvae in 15 min; Petranka, 1983), and likewise, sunfish affect densities of *A. barbouri* larvae in a natural stream (Sih et al., 1992). In fact, *A. barbouri* actively avoid depositing eggs in pools with sunfish present (Kats and Sih, 1992); however, their larvae regularly drift into pools and are consumed by fish predators (Sih et al., 1992). Notably, previous research has not documented a clear relationship between sunfish presence and *A. barbouri* across broad spatial scales. Thus, our findings suggest sunfish may influence local abundance of *A. barbouri* throughout a large portion of their geographic range.

Our findings indicate that maximum sodium concentration was an important predictor of *A. barbouri* abundance. Consequently, sublethal effects of sodium dosing on eggs or larvae may occur within some northeastern and central Kentucky streams. Sublethal doses of sodium have been implicated in slower larval growth (Christy and Dickman, 2002), morphological deformities (Chinathamby et al., 2006), and reduced salinity tolerance (Hua and Pierce, 2013) for some amphibians. The relationship between sodium and *A. barbouri* abundance may be due to road salt applications in early spring coinciding with *A. barbouri* reproduction events. Although road salt concentrations of 145 and 945 mg/L have been shown to disrupt osmoregulation in Spotted Salamander (*Ambystoma maculatum*) egg clutches (Karraker and Gibbs, 2011), sodium concentrations at our sites did not exceed 18.67 mg/L and were highest at urban sites. Because sodium levels were highest at urban streams, and urban streams have many stressors aside from poor water quality (i.e., urban stream syndrome; Walsh et al., 2005), the relationship between *A. barbouri* abundance and sodium at our sites may be confounded. Thus, further research is needed to determine the influence of sodium concentrations on *A. barbouri* in Kentucky streams.

Documenting species occupancy and abundance patterns across a large spatial scale allows for more robust interpretation of the elements that are essential for management decisions. Previous studies conducted at only a few localities identified

factors (i.e., forest fragmentation, water quality, and sunfish presence) that potentially contribute to *A. barbouri* local decline. Our research has corroborated and confirmed the importance of some of these stressors (i.e., sunfish and sodium) and elucidate habitat associations on a larger, 16-county-wide scale. Our findings suggest that *A. barbouri* is widespread throughout northeastern and central Kentucky and that this species has high occupancy among forested and agricultural stream catchments but low occupancy among urban stream catchments. Furthermore, abundance varied with local- and catchment-scale habitat conditions. If managing this species becomes necessary at the state or federal level, preserving low-order streams and minimizing urban development within stream catchments should be a priority. Semlitsch and Bodie (2003) suggest preserving a forested core habitat of 192–339 m with an additional 50-m buffer around streams to maintain populations of most stream-breeding amphibian species. Although upland migration distances for *A. barbouri* have not been thoroughly documented, migrating adults have been observed 400 m from the nearest stream (Petranka, 1998), and genetic research indicates adult *A. barbouri* rarely travel greater than 5 km from their breeding streams (Storfer, 1999). Thus, the recommendation of protecting a forested core habitat plus buffer of 450 m from the stream will likely protect a significant portion of local *A. barbouri* populations. To augment habitat for *A. barbouri* persisting in suboptimal conditions with multiple stressors (i.e., in agriculture catchments with sunfish present or in urban catchments), forested buffers added to the periphery of these streams would provide upland habitat and function to filter stream contaminants before they reach the stream channel (Mander et al., 2005; Polyakov et al., 2005). In urban areas, this may be particularly important because we documented increased levels of sodium, nitrate, and conductivity at urban sites compared with forested sites. Forested buffers adjacent to streams may also improve storm water retention within the catchment, reducing the frequency and intensity of high flow events (Wang et al., 2001) and may prevent pollutants, such as road salt, from entering the stream (Barrett and Price, 2014). We suggest that preserving or restoring forested buffer zones around streams will likely protect *A. barbouri* populations through the maintenance of both their in-stream and terrestrial habitat requirements.

Large-scale field investigations developed using information gleaned from laboratory- and locality-based research allow for a more thorough examination of the drivers of population dynamics. Small-scale locality-based research is important for informing larger scale projects and recovery efforts; however, making inferences based on only small-scale and presence-only data is not recommended. Most small-scale field research is conducted at known localities and can only provide limited interpretation without the incorporation of presumed suboptimal sites where focal species are assumed to be limited or absent. Previous research indicated that *A. barbouri* were locally abundant only in fishless, forested streams, but our findings extend this to include agricultural catchments without sunfish as additional valuable habitat. Our findings will thus inform targeted management strategies for mitigating future population declines.

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