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Effects Of Suburbanizing Landscapes On Reproductive Effort of Vernal Pool-Breeding Amphibians

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Abstract.—**Suburbanization near vernal pools compromises terrestrial habitat and pool water quality thereby potentially reducing amphibian reproductive effort. We examined the effects of tree and impervious cover within 100–1,000 m of vernal pools and of road salt contamination (conductivity) on Wood Frog (*Lithobates sylvaticus*) and Spotted and the Blue-spotted salamander complex (*Ambystoma maculatum* and *A. laterale-jeffersonianum*) reproductive effort at 43 pools across a suburbanization gradient near Bangor, Maine, USA. We studied the relationship between adult Wood Frog body condition and reproductive effort at six pools. Likelihoods of breeding (Spotted and Blue-spotted salamanders) and breeding populations (Wood Frog and Spotted Salamanders) decreased with reduced tree cover within 300–1,000 m and increased conductivity. Wood Frog and Spotted Salamander populations decreased with impervious cover near pools (100 m); however, these responses, along with Blue-spotted Salamander likelihood of breeding, were positively associated with impervious cover within 300–1,000 m. Clutch size increased with male adult Wood Frog size but varied negatively with tree cover within 100 and 300 m and positively with conductivity. Our results demonstrate the complexity of breeding responses to road salt and land cover at differing scales within 1 km of pools. We show that Blue-spotted Salamanders respond to land cover at greater distances than they have been observed to move from breeding pools. Our research adds to the body of literature demonstrating an overall negative effect of suburbanization and suggests that populations may be especially sensitive to impervious cover within 100 m and landscape change within 1,000 m.**

Key Words.—*Ambystoma laterale-jeffersonianum*; *Ambystoma maculatum*; Blue-spotted Salamander; *Lithobates sylvaticus*; reproductive effort; Spotted Salamander; suburbanization; Wood Frog

Introduction

Pool-breeding amphibians in the northeastern United States and southeastern Canada, similar to most groups of amphibians near urbanizing areas worldwide, are threatened by urbanization, including suburbanization, and the resultant habitat loss, fragmentation, and degradation (Windmiller and Calhoun 2008; Baldwin and deMaynadier 2009). These amphibians use vernal pools (fishless, ephemeral pools inundated by snowmelt in early spring) as essential breeding habitat. After breeding they leave vernal pools, then spend the vast majority of their lives in nearby forested areas that provide post-breeding and overwintering habitat (Regosin and Windmiller 2003; Groff et al. 2016). Because these amphibians usually require both aquatic and terrestrial habitats to complete their life cycles, they are sensitive to suburban-associated perturbations in both environments (Gibbs 1998; Homan et al. 2004; Rubbo and Kiesecker 2005).

The conversion of forest to suburban-associated cover types (e.g., impervious surfaces) within 1,000 m of pools has been correlated with breeding population declines for Wood Frog (*Lithobates sylvaticus*) and Spotted Salamander (*Ambystoma maculatum*; Windmiller 1996; Homan et al. 2004; Skidds et al. 2007; Eigenbrod et al. 2008). Although less-studied, members of the Blue-spotted Salamander complex (*A. laterale-jeffersonianum* complex, hereafter referred to as Blue-spotted Salamanders) may have greater population declines in suburban forest fragments than co-occurring Spotted Salamanders (Homan et al. 2007). Furthermore, differences in life history (e.g., breeding frequency) and habitat preferences between Spotted and Blue-spotted salamanders suggest that Blue-spotted Salamanders may have distinct, taxa-specific responses to suburbanization (Regosin et al. 1996; Homan et al. 2007; Hoffmann 2017). Extensive study of Wood Frog and Spotted Salamander breeding in suburbanizing landscapes (Table 1, Appendix Table 1) generally reveals a negative correlation between forest cover, expansion of roads, road salt contamination and pool occupancy, but the effects may be non-linear. For example, the influence of forest removal can differ with spatial scales (Clark et al. 2008; Eigenbrod et al. 2008), and Homan et al. (2004) demonstrated that intensity (i.e., percentages) of forest cover removal within 30–1,000 m corresponded to sharp declines in the likelihood of Wood Frogs and Spotted Salamanders breeding (Appendix Table 1). Although several studies have examined occupancy in

Table 1. Relationships between reproductive response (O = occupancy, C = clutch size, E = embryo size) and breeding pool conductivity and chloride (due to road salt) for Wood Frog (*Lithobates sylvaticus*) and Spotted Salamander (*Ambystoma maculatum*) identified in the literature. States in USA, Provinces in Canada. An asterisk (*) means distance from road negatively correlates with conductivity and chloride, double asterisks (**) means occupied pools had lower conductivity and chloride concentration than unoccupied pools, dagger (†) indicates ≥ mean female body size, and double daggers (††) means roadside embryos were smaller than woodland embryos on average.

Species / urbanization factor	Response	Relationship	Study	State or Province	Road salt contamination (conductivity or chloride)
Wood Frog					
Chloride	O	--	Sanzo and Hecnar 2006	Ontario	0.39–1,030 mg/L
Conductivity	O	--	Hecnar and M'Closkey 1996	Ontario	$\bar{x}$ = 509 µS; 124–3,100 µS
Chloride	O	--	Hecnar and M'Closkey 1996	Ontario	$\bar{x}$ = 38 mg/L; 8–476 mg/L
Chloride	O	--	Collins and Russell 2009	Nova Scotia	Max. occupied: 40 mg/L; Max. unoccupied: 175 mg/L
Road distance *	C	--, †	Brady 2013	Connecticut	Roadside: $\bar{x}$ = about 291 µS; 147 mg/L chloride; woodland: $\bar{x}$ = about 6.7 µS, 3.4 mg/L chloride
Spotted Salamander					
Conductivity **	O	--	Collins and Russell 2009	Nova Scotia	Max. occupied: 30 mg/L; Max. unoccupied: 175 mg/L
Road Distance *	E	+, ††	Brady 2012	Connecticut	Roadside: $\bar{x}$ = 886 µS; 188 mg/L chloride, woodland: $\bar{x}$ = 28 µS; 2.8 mg/L chloride

urbanizing and suburbanizing landscapes, amphibian responses to forest cover can vary by region (e.g., Marsh et al. 2016), and studies examining breeding occupancy in suburbanizing landscapes have not been conducted in the northern Northeastern U.S. Additionally, there are relatively few studies examining breeding population size (abundance), and fewer yet examining clutch size (Appendix Table 1).

Conversion of habitat in terrestrial areas to impervious cover, specifically, introduces a suite of pollutants, most notably road deicing salt in northern areas in North America. Road salt can travel via runoff or vehicle spray into pools and may be implicated in pool-breeding amphibian population declines via reduced survival at aquatic stages, malformation, and exclusion of breeding (Sanzo and Hecnar 2006; Karraker et al. 2008; Collins and Russell 2009; Green and Bailey 2015). Although the effects of road salt on pool-breeding amphibians are predominantly negative, the influence of road salt is complex. For example, road salt is correlated with reduced densities of eggs (clutch/m of pool perimeter) and models predict it can substantially contribute to extirpation of Spotted Salamanders and Wood Frogs from breeding pools near roads (Karraker et al. 2008). However, the distance from road and strength of density dependence can reduce predicted population declines, with potential for Wood Frog larvae to exhibit compensatory or even over-compensatory growth in high-conductivity roadside pools with low larval densities (Karraker et al. 2008; Dananay et al. 2015). Similarly, Spotted Salamander eggs from forest and roadside pools with elevated conductivities (forest: 28 µS mean conductivity; roadside: 886 µS mean conductivity) have lower survival to hatching in roadside pools than forested pools, but in roadside pools, eggs sourced from roadside pools have higher survival than eggs sourced from forest pools when placed in roadside pools with elevated conductivities (Brady 2012). Even when road salt is correlated with larval benefits (e.g., larger body size at pools with high conductivities and low larval densities), it may have delayed negative effects such as reduced post-metamorphic survival (Dananay et al. 2015), with potential impacts on breeding later in life. Another indicator that road salt may negatively influence reproduction is that road salt has also been correlated with indicators of physiological stress in adult breeding male Wood Frogs (Green and Bailey 2015; Hall et al. 2017).

Cover type and road salts in suburbanizing landscapes may also alter clutch size (number of embryos per clutch), via effects on breeding adults. Wood Frogs breeding in roadside pools have been detected to have both greater or similar clutch sizes compared to forest pools, and Spotted Salamanders breeding in roadside pools had smaller clutch sizes (Karraker and Gibbs 2011; Brady 2013). Examining both breeding population size and clutch size may help elucidate the mechanisms of decline in suburbanizing landscapes. Causal linkages between terrestrial conditions, adult pool-breeding amphibian body size, and clutch size has not yet been established, and beginning to examine these relationships in suburbanizing landscapes may provide insights into effects of suburbanization on species such as Wood Frogs, which lay one clutch per female.

Conservation of vernal pool-breeding species typically involves managing terrestrial habitat within some distance from a pool (Calhoun et al. 2005). Although management recommendations are based on documented distances that adult amphibians travel from pools during the non-breeding season, regulations typically permit some development well within the mean distances of terrestrial amphibian movements from pools (Calhoun et al. 2014), and it is not well understood how development within regulated life zones affects the vigor of amphibian populations. Some studies of Blue-spotted Salamander in suburbanizing landscapes have indicated that breeding occupancy is related to forest cover near pools and a post-breeding preference for forests and wet meadows (Ryan and Calhoun 2014; Hoffmann 2017), but largely, the effects of suburbanization near pools have not been well-studied for Blue-spotted Salamander (Appendix Table 1).

Here we examine the effects of both suburban-associated land cover near pools and road salt contamination in pools to compare the relative effects of these common features of suburbanization on Wood Frog, Spotted Salamander, and Blue-spotted Salamander breeding populations. More specifically, we examine two metrics of reproductive effort, the breeding population size as well as clutch size for Wood Frog and Spotted Salamander to better understand how regulated life-zone distances and road salt management strategies may impact population in ways not captured by egg mass counts alone. We did not examine these metrics for the Blue-spotted Salamander complex because high clutch-per-female variability makes their egg and embryo counts unreliable indicators. We hypothesized that reproductive responses are affected by suburbanization as it affects land cover type, vegetation cover, and use of salt for de-icing roads in the area. We predict that reproductive responses would vary negatively with impervious cover and positively with forest cover, with variation in the strength of response corresponding to the intensity of suburbanization. We also anticipated road salt would negatively affect reproductive responses.

Materials and Methods

Study area.—We conducted this study in the greater Bangor area (44°48’8”N, 68°46’13”W) in Maine, USA. The 200 km^2 study area included Bangor, which encompasses 90 km^2 with a population of approximately 33,000, and Orono, Hampden, and Old Town (populations of approximately 7,000–10,000; U.S. Census Bureau. 2011. 2010 Census Demographic Profile. Available from https://www.census.gov/popfinder/?fl=23 [Accessed 03 February 2018]). Developed land uses are primarily residential and commercial with development intensity extremes of nearly 100% impervious surface in downtown Bangor (44°48’8”N, 68°46’13”W) to < 1% impervious surface (e.g., in city conserved lands; Fry et al. 2011). Vernal pools included in the study were embedded in a mixed coniferous-hardwood forest. Each site consisted of a vernal pool and the area within 1,000 m of its high-water mark. We selected sites based on the presence of a Wood Frog and/or Spotted Salamander breeding pool. Some sites were known from a long-term relationship with citizens working on vernal pool conservation (Aram Calhoun, pers. comm.), and we detected others by listening for Wood Frog calling or searching for egg masses in wetland areas identified on aerial photographs.

Site description.—Each site consisted of a Wood Frog and/or Spotted Salamander breeding pool and the area within 1,000 m of the high-water mark. We studied 35, 41, and 36 sites (43 total) in 2014, 2015, and 2016, respectively. We selected sites to represent the range of development intensity at which vernal pool-breeding amphibian reproduction occurred in the greater Bangor area. Sites had 0–35% impervious cover within 100 m, 0–38% within 1,000 m, and 0–100% tree canopy over pools. Pool area at spring high water ranged from 24–9,978 m^2.

Egg mass and embryo counts.—We used counts of egg masses and embryos per clutch (clutch size) to indicate reproductive effort, with egg mass counts representing breeding population size or breeding presence. Following the apparent peak of breeding (based on weekly monitoring), two observers conducted egg mass counts during precipitation-free daylight hours for Wood Frog, Spotted Salamander, and Blue-spotted Salamander at 35, 37, and 36 pools (44 total) in 2014, 2015, and 2016, respectively (See Appendix for additional detail regarding egg count methods). In 2015 and 2016, we counted the embryos in a subset of Wood Frog egg masses in 22 and 27 pools (28 total) and of Spotted Salamander egg masses in 27 and 22 pools (29 total; 38 sites between both species). Even though each female Spotted Salamander can produce multiple egg masses (typically a larger, primary mass and one or two secondary, smaller egg masses; Hunter et al. 1999), and thus lead to overestimates, these counts may still provide useful information. We counted and photographed embryos in a minimum of five egg masses for a species at a site using techniques described in Karraker (2007). We counted embryos using ImageJ (Schneider et al. 2012).

Adult Wood Frog measurements.—We captured and measured adult male Wood Frogs to examine the correlation between adult size and reproductive effort.

We did not capture enough females across pools to incorporate their sizes into analyses. Male size can be positively associated with increased breeding success (especially in male-skewed populations; Berven 1981) and may also be a suitable proxy for breeding female size because adult body size in both sexes can respond similarly to conditions such as juvenile population size (Berven 2009). We used minnow traps to capture adult male Wood Frogs in nine breeding pools from 13–24 April 2016. We weighed frogs, measured snout-vent length (SVL), and toe-clipped new captures to prevent resampling. Given high breeding fidelity to their natal pool (Berven and Grudzien 1990; Vasconcelos and Calhoun 2004), we assumed that > 80% of males were sampled at their natal pool.

Site characteristics.—We used ArcView GIS 10.2 (Esri, Redlands, California, USA) and the Maine Land Cover Dataset (2004 all land use; 2011 impervious surface; 5 m resolution) to quantify the percentage imperviousness (IMP) and tree (TREE) cover within 100, 300, 600, and 1,000 m from pool high water marks. We digitized tree cover from aerial photographs in disturbed and undisturbed areas, thus we cannot assume that tree cover represents forest, but tree cover in either area likely corresponds with ground shading and cooling (Leuzinger et al. 2009). We used water probes (Hach Company, Loveland, Colorado, USA) to sample specific conductance (SPCOND), which indicates road salt contamination, at 43 pools between 2 May to 16 June 2014–2016. On each sampling date, we collected, and within minutes tested, 1 L of surface water about 1 m from the edge at each of three equidistant points around the perimeter of each pool. We averaged SPCOND by day to represent salt concentration throughout seasons when amphibians use pools. We sampled > 1 y at 31 sites.

Analyses.—In all analyses of effective population size, we only included maximum egg mass counts from pools where the focal species was detected at least one year. We examined the relationships between site characteristics (land cover and specific conductivity) and reproductive effort indicated by egg masses (detected effective population size of Wood Frog and Spotted Salamanders; likelihood of detected breeding of spotted and Blue-spotted Salamanders) by site-year using Random Forest Analyses (RFA) of Classification and Regression Trees (CART) in the computer package randomForest (Liaw and Wiener 2002). We also examined differences in conductivity between pools with and without detected breeding using individual Mann-Whitney tests ($\alpha = 0.05$) for Spotted and Blue-spotted salamanders. We bootstrapped with replacement to build 10,000 regression trees (Random Forest error stabilized at approximately 1,000–2,000 trees for each response variable), using 2/3 of the data at each iteration. We calculated explanatory variable importance (by rank) using the mean percentage decrease in accuracy resulting from removal of each variable. We created Partial Dependence Plots (PDP; Ishwaran and Kogalur 2014) that examine the marginal effects of predictor variables while holding all other predictors at average values (Friedman 2001; Cutler et al. 2007). Because PDPs display general trends, all reported values are approximate.

To examine the effects of land cover type at 100-1,000 m and specific conductivity on clutch size, we fit linear models that included one predictor as well as Year (see Appendices: Additional Analytical Methods), using nlme (Pinheiro et al. 2017). We then ranked models using AIC adjusted for small sample size (AICc) using AICcmodavg (Mazerolle 2017). We considered models ΔAICc < 2 that ranked above the null model to be plausible (Burnham and Anderson 2002). If > 1 model had ΔAICc < 2, we tested additive models that included all combinations of covariates in these highly ranked models. We examined the 85% confidence intervals (Arnold 2010) of covariates in all plausible models to determine effect. Prior to fitting univariate models, we determined optimal model structure by comparing model fit among full models (including all land cover variables and specific conductivity as predictors) with no random effect and that had Site or Year as a random effect. We used the structure from the highest-ranking (ΔAIC = 0) model with the simplest structure; i.e., we selected models without a random structure over those with a random structure if both were ΔAICc < 2 from each other. See Appendix for additional analytical methods regarding embryo detection probabilities.

We examined the effect of breeding population size (egg mass count) on clutch size across all years for Wood Frog and Spotted Salamander and the effects of median adult SVL and median size-adjusted mass (residual of natural log-transformed mass plotted against natural log-transformed SVL) on 2016 clutch size for Wood Frog using linear regression. We removed one site from the regression because we observed only two egg masses in 2016. For both sets of models, we initially compared structure between models without a random effect and with Site as a random effect. We then examined the effects of significant ($P < 0.05$) predictors within the highest-ranking models (ΔAIC = 0). All analyses were conducted using R statistical software (R Core Team 2016).

Results

Detected breeding.—We detected up to 426 and 391 egg masses of Wood Frogs and Spotted Salamanders, respectively, per site-year, and detected breeding at

Table 2. Reproductive responses to conditions in a developing landscape in greater Bangor, Maine, USA, modeled for Wood Frog (*Lithobates sylvaticus*), Spotted Salamander (*Ambystoma maculatum*), and Blue-spotted Salamander (*A. laterale-jeffersonianum*). Sample sizes (n) are for pools with the number of individuals found in parentheses. Observed range and medians are untransformed values. Egg mass counts are from pools where breeding presence was detected in at least one year.

Response	n	Range (median, mean)
Maximum egg mass count		
Wood Frog	44 (n/a)	0–426 (22.5, 40.8)
Spotted Salamander	34 (n/a)	0–391 (8.0, 36.2)
Blue-spotted Salamander	27 (n/a)	--
Embryos per egg clutch		
Wood Frog	27 (453)	120–1,469 (642.0, 650.8)
Spotted Salamander	26 (744)	4–224 (97.0, 95.6)

108, 82, and 54 site-years for Wood Frogs, Spotted Salamanders, and Blue-spotted Salamanders, respectively (Table 2). We detected Spotted Salamander and Blue-spotted Salamander breeding in pools with ≤ 646 µS, ≤ 34% impervious cover within 100 m, ≤ 32% and ≤ 27% (respectively) impervious cover within 1,000 m, ≥ 36% and ≥ 8% tree cover within 100 m, and ≥ 17% and ≥ 29% tree cover within 1,000 m. Twenty-seven sites had differences of ≤ 40 µS between years and only four sites had a difference > 150 µS between years (161–872 µS).

Wood Frog breeding population size, as indicated by egg mass counts, varied by cover types at multiple scales, with top-ranked predictors at 100–1,000 m (Fig. 1a). Population size corresponded negatively to suburban-associated land cover at 100–600 m. Small increases (0–5%) in impervious cover near pools (100 m) predicted reduced population size and increases in tree cover > 50% within 600 m associated with larger population sizes (Fig. 2). However, 0–38% impervious cover at larger scales (600 and 1,000 m) correlated positively with population size (Fig. 2).

Spotted Salamander breeding population size and the likelihood of detected breeding were generally negatively associated with suburbanization. Cover types, at multiple scales, predicted both response variables with the most important predictor for each operating at relatively small scales (100 m for population size; 300 m for likelihood of breeding; Fig. 1b–c). Small increases (0–7%) in impervious cover near pools (100 m) predicted Spotted Salamander population decreases, but small increases (0–7%) in impervious cover at 600 m predicted population increases. Tree cover positively corresponded with population size (1,000 m) and likelihood of detected breeding (300–600 m), with steep declines in population size predicted with tree cover losses at 1,000 m between 60–80% and in

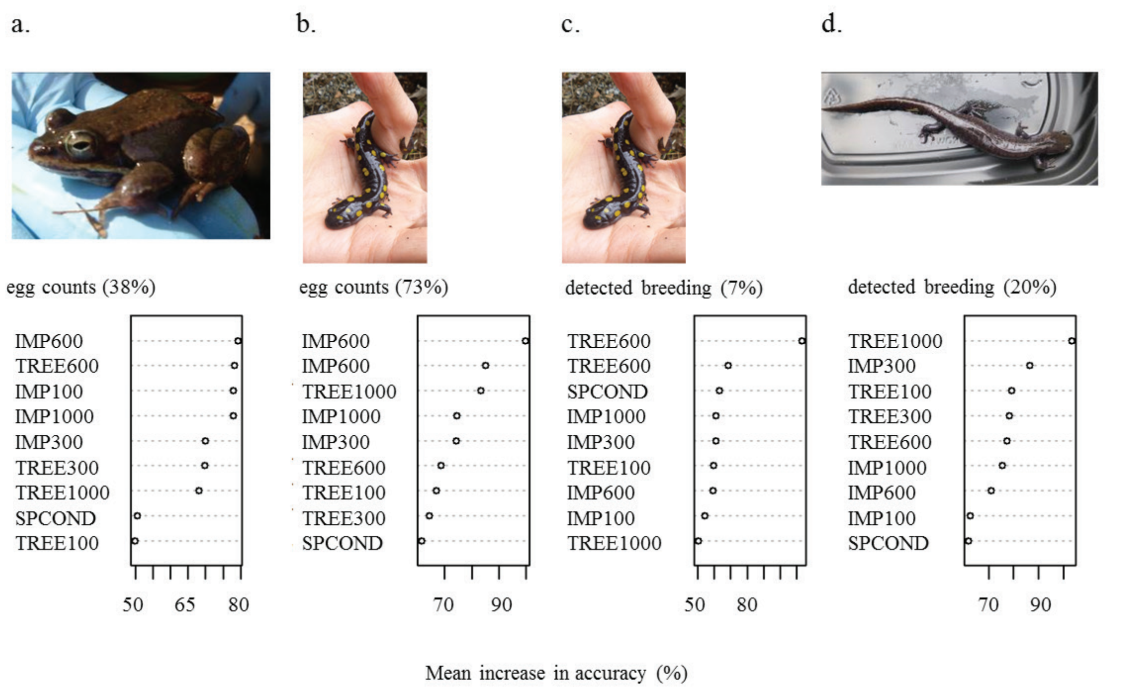

Figure 1. Variable importance plots from random forest models (a, b) for ln (mean egg mass count +1) of Wood Frog (*Lithobates sylvaticus*) and Spotted Salamander (*Ambystoma maculatum*) regression trees; and from classification trees (c, d) of presence of *A. maculatum* and Blue-Spotted Salamander (*A. laterale-jeffersonianum*). Plots show the rank-order of explanatory variables along the y-axis and the percent average increase in mean square error when the values of the given variable are randomized while all others are held constant along the x-axis. Parenthetical percentages represent the variation explained for egg mass counts (a, b) and out of bag (OOB) estimate of error rate for detected breeding (c, d). Variables along the y-axis are impervious (IMP) and tree cover (TREE) within 100, 300, 600, and 1,000 m and specific conductivity (SPCOND). (Photographed by Carly Eakin).

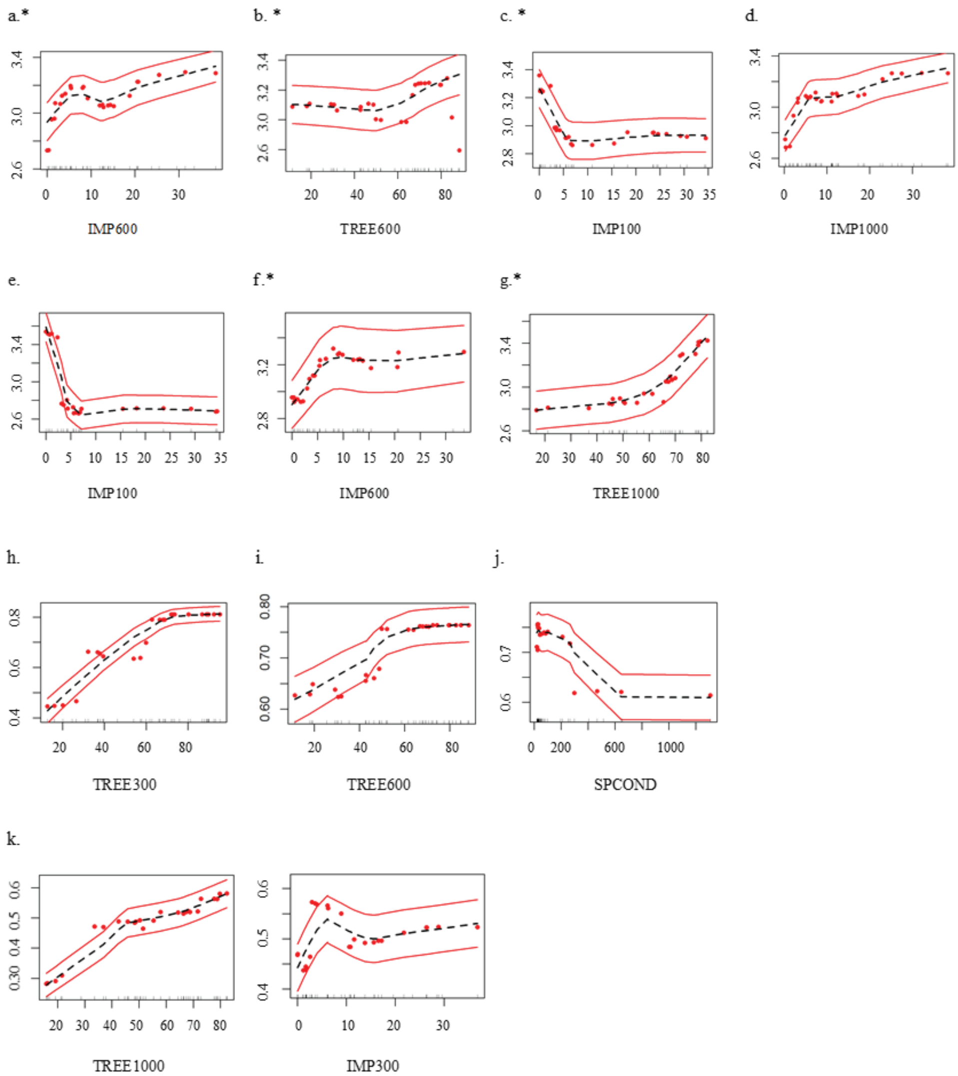

Figure 2. Partial dependence plots from random forest predictions of natural log-transformed Wood Frog (*Lithobates sylvaticus*) egg mass counts (a-d), natural log-transformed Spotted Salamander (*Ambystoma maculatum*) egg mass counts (e-g) and likelihood of breeding presence (h-j), and likelihood of Blue-spotted Salamander (*A. laterale*) breeding presence (k-l) plotted against impervious (IMP) and tree cover (TREE) within 100, 300, 600, and 1,000 m. In a partial dependence plot of marginal effects, only the relative values (and not the absolute values) of predicted responses can be interpreted. The black dashed line corresponds to a lowess smoothed line representing the partial dependence between an explanatory variable and response. The dashed red lines indicate a smoothed error bar of +/-two standard errors. The red dots indicate the partial values used to fit the lowess function.

breeding likelihood predicted with losses of tree cover at 300 and 600 m below 70% and 50%, respectively (Fig. 2). Increases in specific conductivity up to 650 µS corresponded with reductions in likelihood of breeding (Fig. 2). Conductivity between pools where breeding was and was not detected differed significantly (U = 232, P = 0.05).

Tree cover within 1,000 m was the most important predictor for the likelihood of detected breeding for Blue-spotted Salamander and was positively correlated with breeding likelihood up to at least 82% (Fig. 1d and 2). Impervious cover within 300 m, however, generally positively correlated with breeding likelihood, with the strongest effect predicted between 0–7% impervious cover (Fig. 2). There were no significant differences in conductivity between pools where breeding was and was not detected (U = 206, P = 0.72).

Clutch size.—We counted embryos in 453 Wood Frog and 744 Spotted Salamander egg masses. Wood Frog egg masses had 120–1,469 embryos (median = 642) and Spotted Salamanders had 4–224 embryos (median = 97) per egg mass (Table 2, Appendix Tables 2 and 3). There were differences between years for Wood Frog

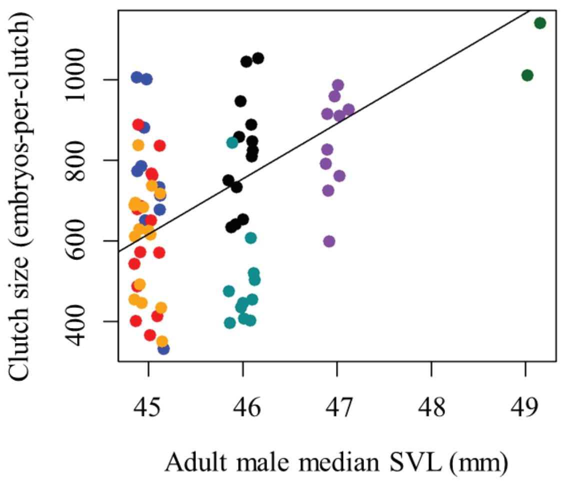

Figure 3. Clutch size in relation to median size of male Wood Frogs (*Lithobates sylvaticus*) at breeding pools in 2016. Noise (i.e., x-axis jitter) added for legibility; SVL measurements are integers. Colors represent pools (n = 7), and regression line excludes two outliers, SVL = 49 mm.

and Spotted Salamander clutch size (Wood Frog: Site as a random effect; $F_{1,425}$ = 10.88, P = 0.001; Spotted Salamander: random effect not retained; $F_{1,742}$ = 10.02, P = 0.002).

Wood Frog clutch size decreased with tree cover within 100 and 300 m (Tables 3 and 4). Conductivity had a positive effect on Wood Frog clutch size, with increases of 100 µS in conductivity predicted to increase Wood Frog clutches by 13 embryos (Table 4). There was no effect of site characteristics on Spotted Salamander clutch size nor of the effective breeding population size on clutch size (Wood Frog, Site as a random effect, $F_{1,424}$ = 1.86, P = 0.174; Spotted Salamander, no random effect, $F_{1,741}$ = 0.622, P = 0.431). Median male adult SVL had a positive effect on clutch size, although there was no significant effect of size-adjusted mass (no random effect; SVL: $F_{1,70}$ = 7.41, P = 0.008, Fig. 3; Mass: $F_{1,70}$ = 3.10, P = 0.083; See Appendix Table 4 for summarized adult female size measurements).

Discussion

Our results suggest that decreases in tree cover (including canopy cover in suburbanizing areas as well as forest) within 1,000 m, increases in impervious cover within 100 m, and increases in road salt contamination negatively affect reproductive effort for Wood Frog, Spotted Salamander, and Blue-spotted Salamander. Increased specific conductivity and/or reductions in tree cover were associated with reduced breeding likelihoods and/or fewer egg masses for the three studied species. Similarly, egg mass counts for Wood Frog and Spotted Salamander greatly decreased with small increases (0–5%) in impervious cover within 100 m. Additionally, we detected declines in the likelihood of Spotted Salamander breeding corresponding to tree cover at < 70% within 300 m and < 50% within 600 m.

Table 3. Ranking of Wood Frog (*Lithobates sylvaticus*) models that ranked < 2 ΔAICc and ranked above the null model. Null models are included for reference. Observations are nested by Site (random effect). The abbreviation K = number of parameters, AICc = Akaike Information Criterion adjusted for small sample size, ΔAICc = AICc difference with top ranked model, w = relative model weight (0–1), and LL = log-likelihood estimate.

	K	AICc	ΔAICc	w	LL
TREE100 + Year + Site	5	5881.76	0	0.14	-2935.88
SPCOND + Year + Site	5	5882.02	0.26	0.12	-2936.01
TREE300 + Year + Site	5	5882.13	0.37	0.12	-2936.07
Year + Site (Null)	4	5882.26	0.5	0.11	-2937.13

The association of smaller breeding populations of our three study species (or a reduced likelihood of breeding) with sparse forest cover support similar findings in other studies. Windmiller et al. (2008) documented substantial declines in Wood Frog, Spotted Salamander, and Blue-spotted Salamander (complex) breeding emigration after 41% forest removal within 300 m of a pool; and Homan et al. (2004) detected reduced forest cover negatively correlated with Spotted Salamander breeding occupancy at all studied scales (30–1,000 m) with thresholds of declines in occupancy at 30% and 41% forest cover within 100 and 500 m, respectively. The substantial declines we detected may have corresponded with greater forest cover than those detected by Homan et al. (2004) because they selected study pools using remote sensing whereas we only studied pools with confirmed breeding presence of Wood Frog or Spotted Salamander.

Lower Wood Frog and Spotted Salamander breeding populations associated with relatively little impervious cover within 100 m of pools suggest that these species are especially sensitive to suburban land conversion near pools. Additionally, these results align with studies that demonstrate the importance of conserving forested areas near pools for adult habitat of pool-breeding amphibians. For example, 40–60% of Wood Frogs and Spotted Salamanders overwinter < 100 m away from breeding pools (Regosin et al. 1996; Regosin & Windmiller 2003) and others have found a mean hibernacula distance of approximately 125

Table 4. Estimates, standard errors, and 85% confidence intervals (CIs) of covariates of Wood Frog clutch size for models with < 2ΔAICc and that rank above null models. Covariates are listed in order of AICc of their respective model. The abbreviation β estimate = parameter estimate, SE = standard error, and CI = 95% confidence interval.

	β estimate	SE	Lower CI	Upper CI
TREE100	-1.36	0.844	-2.61	-0.110
SPCOND	0.128	0.0845	0.00323	0.253
TREE300	-1.39	0.937	-2.78	-0.00317

m and a maximum distance of 317 m from breeding pools (Groff et al. 2016). Moreover, forest cover is vital for movements made outside the breeding season; ≥ 2/3 of Spotted Salamander movements occur within 200 m (Regosin et al. 1996), and 95% of adult Blue-spotted Salamanders occur within 152 m of pools (Ryan and Calhoun 2014). Increases in impervious surfaces almost assuredly remove the burrows and uncompacted substrates on which these species rely for cover and overwintering (Madison 1997; Regosin et al. 2003). In areas where lightly compacted yet vegetated areas (e.g., lawns) are typically associated with impervious cover, as is the case in the Greater Bangor area, more impervious cover likely corresponds to even greater losses of areas where burrows and uncompacted soil can be found. Given the suburban character of our study area, much of the impervious cover was likely roads and driveways, which can also reduce amphibian populations directly via road mortality (Fahrig et al. 1995; Gibbs and Shriver 2005). Thus, the negative effects of impervious cover near pools we observed may have multiple causes.

The reduced likelihood of Spotted Salamander breeding in pools with higher specific conductivity supports other studies and experiments indicating that road salt contamination may reduce or eliminate breeding populations via effects at aquatic stages (Turtle 2000; Karraker et al. 2008). This suggests that preventing road salt contamination, especially near roads (Karraker et al. 2008), may be necessary for maintaining Spotted Salamander populations in areas of even relatively low-intensity suburbanization. It is possible that our detected effect of specific conductivity may be confounded by road mortality, which can contribute to extirpation of breeding populations of Spotted Salamanders (Gibbs and Shriver 2005) and may encompass the total effects of nearby roads rather than the effects conductivity alone.

Counterintuitively, impervious cover at larger scales (300–1,000 m) was positively associated with Wood Frog and Spotted Salamander breeding population size along with Blue-spotted Salamander likelihood of breeding. It is possible that this relationship could be an artifact of the particular landscape we studied. For example, the distribution of pools and suburbanization may have some underlying, similar geomorphic driver. Another possible explanation is that the intermediate level of disturbance in our study area has some positive effects on these amphibians via an unknown mechanism (e.g., higher quality food sources). A more plausible explanation for this relationship is the potential loss of breeding pools resulting in displaced adults and first-year breeding recruits consolidating breeding in remaining pools. The idea that isolation of suitable breeding pools can increase breeding population size in those pools has been proposed by others (Calhoun et al. 2003; Baldwin et al. 2006; Veysey et al. 2011). Moreover, genetic analyses for Wood Frogs and Spotted Salamanders have indicated that populations become more isolated with increased road density within 1 km of pools (Jared J. Homola, pers. comm.). This consolidation of breeding effort could lead to egg mass count-driven population assessments to inappropriately conclude that populations were stable or even benefiting from suburbanization. Thus, we suggest that future studies examining the impacts of suburbanization on an egg mass count-derived response consider amphibians from sets of pools as one population. This follows the recommendations of Petranka et al. (2004), Zamudio and Wieczorek (2007) and Veysey et al. (2011) to treat clusters of pools as single demographic units. Additionally, we suggest assessing terrestrial stage habitat quality, body condition, and survival to better identify responses in suburbanizing landscapes.

The clutch sizes we observed of 120–1,469 for Wood Frogs and 4–224 for Spotted Salamanders were similar to those in other locations throughout the northeastern U.S. (Wood Frog: about 300–1,250 embryos per clutch in Maryland, Berven 1988; mean = 664 embryos per clutch in Connecticut, Halverson et al. 2006; 514–1,012 in New York, Karraker and Gibbs 2011; about 800 in Connecticut, Brady 2013; Spotted Salamander: 7–228 in New York, Karraker and Gibbs 2011; about 100 in Connecticut, Brady 2012). The effects of tree cover near pools (100–300 m) and specific conductivity on Wood Frog clutch size, however, suggest that clutch size may increase with suburbanization intensity. Because female Wood Frog size has been positively correlated with clutch size (Howard and Kluge 1985; Berven 1988), the effect of suburbanization on clutch size may be linked to female body size. The positive correlation between adult male SVL and clutch size in a subset of study pools supports this relationship, if one assumes terrestrial habitat quality has a similar effect on the size of males and females. Female body size has also been positively correlated with clutch size for Spotted Salamander as well as for two other congeners in temporary pools (Kaplan and Salthe 1979; Woodward 1982). Thus, the lack of detected effect of land cover conversion or specific conductivity on Spotted Salamander clutch size in our study suggests no difference in female body size across the studied suburbanization gradient.

Larger clutch sizes with increased suburbanization aligns with the observation by Brady (2013) that clutch size increased with female body size at a greater rate in high-salinity, roadside pools compared to low-salinity, woodland pools (but see Karraker and Gibbs 2011); however, greater embryo mortality was detected in roadside pools (Brady 2013) and sodium chloride-contaminated stormwater management pools (Snodgrass et al. 2008). Thus the outcome of larger clutch sizes with increasing suburbanization intensity may not increase the breeding population size. It is also plausible that declines in breeding population size in suburbanizing areas may reduce competition for resources among the remaining adults, allowing these adults to grow larger and potentially produce larger clutches (Harper and Semlitsch 2007; Patrick et al. 2008). We suggest further study to better understand the population implications of clutch size differences in suburbanizing areas.

Our study affirms past research demonstrating an overall negative effect of suburbanization on pool-breeding amphibians while highlighting the nuance of reproductive responses to suburban-associated land cover at scales within 1 km of pools and road salt within-pool. Although we show that road salt and relatively low intensities of impervious cover can have positive associations with reproductive responses (Wood Frog clutch size), our work is correlative and does not definitively indicate causation. We encourage future work to thoroughly examine possible mechanisms for these responses, such as post-metamorphic density-dependence or habitat quality. We also stress that we did not examine other potential responses to suburbanization that may affect population persistence, such as age structure (Semlitsch et al. 1988; Berven 1990; Jennette et al. 2019), physiological stress (Homan et al. 2003; Hall et al. 2017), and connectivity with other populations (Compton et al. 2007).

Our study also adds to the small body of literature examining the effects of suburbanization on Blue-spotted Salamander reproduction and expands the scale at which breeding occupancy corresponds to suburbanization (1,000 m compared to 300 m examined by Windmiller et al. 2008). The relatively strong effect of forest cover at 1,000 m compared to smaller scales within our study area suggests that these salamanders may be particularly sensitive to land conversion at large scales, especially when considered in the context of previous work showing no effect of forest cover within 200 m on breeding occupancy (Hoffmann 2017) and population declines following the conversion of forest to residential cover within 300 m (Windmiller et al. 2008). This scale of effect is greater than that expected by the distances others have detected Blue-spotted Salamanders moving from breeding pools (410 m for unisexuals, Hoffmann et al. 2018; and 281 m for sexually breeding diploids, Ryan and Calhoun 2014). Because we did not differentiate between occupancy of unisexuals and sexually breeding individuals, our results may describe the response of only one of these groups.

Because suburban-associated cover types near pools have the most consistent negative effects on egg mass numbers and we detected effects at relatively low levels of cover, maintaining forest cover and other undisturbed areas within 100 m of breeding pools is likely critical to avoid direct reductions in breeding population size and breeding occurrence. Furthermore, conservation of pool-breeding amphibians in suburbanizing landscapes may be best served by taking a landscape-level view that connects breeding pools with high-quality non-breeding habitat in close proximity (Compton et al. 2007; Sawatzky et al. 2019). We encourage further research that examines how landscape configuration contributes to pool-breeding amphibian responses. Additionally, our results suggest that the effects of conductivity on clutch size may not contribute to breeding population size, but we note that other effects of road salt (e.g., increased larval mortality) may contribute to these declines.

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Appendix

Additional egg count methods.—We disturbed eggs as little as possible, avoided lifting from the water or detaching from substrates. In areas safely accessible while wearing chest waders (slightly > 1 m deep), observers walked transects ≤ 3 m apart, paying extra attention to vegetation and downed woody material where amphibians attach eggs. Although some pools had deep, inaccessible areas, underestimation was likely minimal for Wood Frog as they commonly deposit eggs at or near the surface, making the eggs highly visible, and for Spotted Salamander which deposit the majority of eggs (> 91%) <1 m deep (Windmiller 1996). Blue-spotted Salamander attach eggs to similar substrates as Spotted Salamanders (e.g., submerged sticks and vegetation) as well as along pool bottoms, potentially reducing our likelihood of detection for these salamanders (Uzzell 1967; Gilhen 1974). In 2014, double-observers counted egg masses for greater detection, but only one count was recorded per pool. In 2015 and 2016, we used dependent double-observer counts, following Grant et al. (2005) where the second observer was aware of the eggs detected by the first observer, but not vice versa. Thus the second observer detected at least as many eggs as the first observer. We used Blue-spotted Salamander egg mass counts to indicate breeding presence, not to indicate breeding population size because of the high variability in how many egg masses are laid by a single female (Wilbur 1971).

Additional analytical methods.—In analyses, we did not account for detection probabilities because n-mixture models (Fiske and Chandler 2017) performed poorly with improbable detection covariate values (e.g., greater pool sizes had greater detection probabilities) and unrealistically high estimates of egg masses (e.g., 150–1,000% of the counted masses in approximately 1/3 of pools). Random Forest analyses (RFA) of classification and regression trees (CART) were used for analyses of effective population size and likelihood of occupancy. RFA avoids over-fitting models because the process constructs classification trees for each response variable and selects the dominant classification structure (Breiman 2001). CARTs allow for high correlation of covariates and identify the relative importance of covariates while holding all other variables at their mean. By using multiple years of data from sites as separate observations we captured among-year variation, a well-documented trait of breeding population size of these amphibians (Berven 2009; Capps et al. 2015). We natural-log transformed abundance so the model (CART) would be unbiased by high and low egg mass counts. RFAs were particularly appropriate because population size and likelihood of breeding likely does not respond linearly to land cover types and because there was high correlation ($r > 0.59$) among all land cover type variables. Additionally, CART have been found to be more accurate than negative binomial regression models of count data, which can be used to account for the overdispersion and non-normal distribution of count data (Wah et al. 2012).

Prior to modeling the effects of site characteristics on clutch size (counts of embryos per clutch), we verified the detection probability using embryos from 5 Wood Frog and 19 Spotted Salamander egg masses. Two or more observers counted each egg mass 2–7 times (Spotted Salamander median = 2, total = 52; Wood Frog median = 4; total = 23). We assumed that undercounting was more likely than over-counting because of glare and superimposed images of embryos in the photos, so we compared the maximum count to all other counts (equal or fewer embryos than the maximum) to determine the proportion of embryos detected. Detection was relatively high (median non-maximum count = 98%; Wood Frog: 86-100%; Spotted Salamander: 81-100%), so we used the counted number of embryos per egg mass for clutches counted once and used the median count for clutches counted multiple times.

Prior to the modeling, we also examined the effect of Year on clutch size for Wood Frog and Spotted Salamander. Using data only from site-years where we counted embryos in ≥ 5 egg masses, we regressed clutch size on year. We initially compared model structure between models without a random effect and with Site as a random effect using Akaike's information criterion (AIC). We then examined the effects of Year in the model with the highest-ranking model (ΔAIC = 0) for both species. If Year had a significant effect ($P < 0.05$), we included it as a fixed effect in all further models.

Appendix Table 1. Literature review of landscape level investigations of the relationships between urban/suburban cover factors (e.g., loss of forest, expansion of roads) and reproductive response (O = occupancy, A = abundance) for Wood Frog (*Lithobates sylvaticus*), Spotted Salamander (*Ambystoma maculatum*), and Blue-spotted Salamander (*A. laterale-jeffersonianum*). When land cover variables were studied at multiple spatial scales, we report distance range. An asterisk (*) means area includes Vermont, New Hampshire, Massachusetts, New York, Pennsylvania, Maryland, Virginia, North Carolina, South Carolina, Georgia, Florida, Missouri, and Minnesota.

Species urbanization factor	Response	Relationship	Study	State or Province (USA, CA)	Land cover characterization
Wood Frog					
Forest cover (100 m to 2 km)	O	+, 100 m	Eigenbrod et al. 2008	Ottawa	Developing rural areas
Forest cover (200 m to 1 km)	O	+, 200 m to 1 km	Porej et al. 2004	Ohio	Developing, agriculture-dominated
Forest cover (1 ha grid)	O	+, Occupancy at > 30% forest cover	Gibbs 1998	Connecticut	Rural to urban; 8–94% forest cover
Forest cover (30 m to 1 km)	O	+, 30 m to 1 km	Homan et al. 2004	Massachusetts	Rural to urban; about 10–90% forest cover
Forest (1 km)	O	+	Rubbo and Kiesecker 2005	Pennsylvania	Rural to urban; $\bar{x}$ = 38-86% forest cover by development class
Wet forest cover (100 m to 2 km)	O	+, 300 m to 2 km	Clark et al. 2008	Massachusetts	Suburban to urban
Forest cover (1 km)	A	+, 1 km	Skidds et al. 2007	Rhode Island	Study area: 57% forest, 15% residential, 8% agriculture
Impervious cover (300 m to 10 km)	O	No effect	Marsh et al. 2016	Eastern and Central U.S.*	Rural to suburban; 2–37% impervious cover, 25–98% forest cover
Road density (300 m to 10 km)	O	-, 300 m to 10 km	Marsh et al. 2016	Eastern and Central U.S.*	Rural to suburban; 2–37% impervious cover, 25–98% forest cover
Road length (100 m to 2 km)	O	-, 1 km	Clark et al. 2008	Massachusetts	Suburban to urban
Building number (1 km)	A	-, 1 km	Skidds et al. 2007	Rhode Island	Study area: 57% forest, 15% residential, 8% agriculture
Residential cover (1 km)	A	-, 1 km	Skidds et al. 2007	Rhode Island	Study area: 57% forest, 15% residential, 8% agriculture
Forest conversion to residence (300 m)	A	-	Windmiller et al. 2008	Massachusetts	Suburban
Spotted Salamander					
Forest cover (200 m to 1 km)	Oc	+, 200 m	Porej et al. 2004	Ohio	Developing, agriculture-dominated
Forest cover (1 km)	A	+, 1 km	Skidds et al. 2007	Rhode Island	Study area: 57% forest, 15% residential, 8% agriculture

Appendix Table 1 (continued). Literature review of landscape level investigations of the relationships between urban/suburban cover factors (e.g., loss of forest, expansion of roads) and reproductive response (O = occupancy, A = abundance) for Wood Frog (*Lithobates sylvaticus*), Spotted Salamander (*Ambystoma maculatum*), and Blue-spotted Salamander (*A. laterale-jeffersonianum*). When land cover variables were studied at multiple spatial scales, we report distance range. An asterisk (*) means area includes Vermont, New Hampshire, Massachusetts, New York, Pennsylvania, Maryland, Virginia, North Carolina, South Carolina, Georgia, Florida, Missouri, and Minnesota.

Species urbanization factor	Response	Relationship	Study	State or Province (USA, CA)	Land cover characterization
Forest conversion to residence (300 m)	A	-	Windmiller et al. 2008	Massachusetts	Suburban
Forest cover (1 ha grid)	O	+, only occur at < 30% forest cover	Gibbs 1998	Connecticut	Rural to urban; 8–94% forest cover
Forest cover (30 m to 1 km)	O	+, 30–1000 m	Homan et al. 2004	Massachusetts	Rural to urban; about 10–90% forest cover
Forest cover (200 m)	O	+	Hoffmann 2017	Maine	Rural to suburban
Residential area (100 m to 2 km)	O	+, 100 m	Clark et al. 2008	Massachusetts	Suburban to urban
Residential area (100 m to 2 km)	A	+, 100-500 m	Clark et al. 2008	Massachusetts	Suburban to urban
Road length (100 m to 2 km)	A	-, 100-500 m	Clark et al. 2008	Massachusetts	Suburban to urban
Blue-spotted Salamander					
Forest cover (200 m)	O	No effect	Hoffmann 2017	Maine	Rural to suburban
Forest conversion to residence (300 m)	A	-	Windmiller et al. 2008	Massachusetts	Suburban

Appendix Table 2. Wood Frog (*Lithobates sylvatica*) clutch size (number of embryos per egg mass) by site-year for 45 pool-years (27 pools) in greater Bangor, Maine, USA. Summary statistics are based on clutch sizes of egg masses counted once and median clutch size for egg masses with >1 count, n = number of clutches counted in a pool.

Site	2015 n	min	max	median	2016 n	min	max	median
B01	--	--	--	--	7	686	1,110	870
B08	20	537	959	745	10	333	1,006	753
B10	4	483	659	559	10	395	715	516
B12	4	788	1035	900	10	599	987	868.5
B13	2	466	480	473	16	400	786	534
B17	--	--	--	--	9	468	619	511
B18	2	497	588	543	13	642	1,110	776
B23	9	590	948	801	4	1011	1,140	1,076
H01	13	345	619	450	14	367	889	612
H02	16	403	1004	779	11	425	1,469	872
OR08	4	394	664	591	1	247	247	247
OR09	7	455	774	599	--	--	--	--
OR11	1	366	366	366	15	351	837	626
OR12	15	308	600	511	10	534	828	709
OR16	32	437	1029	643	13	634	1,053	824
OR19	--	--	--	--	9	180	828	643
OR21	21	395	945	667	11	397	844	455
OR24	--	--	--	--	5	534	891	549
OR25	20	398	879	642	11	543	903	693
OR26	1	739	739	739	9	318	882	807
OR27	--	--	--	--	11	389	1,130	826
OT09	11	120	554	258	5	355	503	393
OT10	15	378	766	571	11	272	703	472
OT12	12	499	972	801	16	537	899	761
OR15	8	524	963	687	--	--	--	--
OR22	12	323	606	422	--	--	--	--
OR28	20	211	911	543	--	--	--	--

Appendix Table 3. Spotted Salamander (*Ambystoma maculatum*) clutch size (number of embryos per egg mass) by site-year for 43 pool-years (26 pools) in greater Bangor, Maine, USA. Summary statistics are based on clutch sizes of egg masses counted once and median clutch size for egg masses with > 1 count, n = number of clutches counted in a pool.

Site	2015 n	min	max	median	2016 n	min	max	median
B08	8	12	101	54	--	--	--	--
B17	22	46	202	125	26	35	161	74
B20	29	34	146	73	19	39	175	99
B22	28	4	150	76	20	7	170	83
B25	30	12	210	118	--	--	--	--
B26	15	5	176	117	20	20	210	111
H01	17	14	173	91	30	28	154	108
H02	8	39	134	74	18	13	185	77
OR07	10	14	147	86	--	--	--	--
OR08	9	9	221	99	--	--	--	--
OR11	16	21	156	109	20	13	153	108
OR12	15	23	178	81	20	9	199	125
OR16	36	10	173	89	20	11	169	96
OR17	15	10	206	88	20	33	210	110
OR21	5	47	122	96	16	30	124	94
OR24	--	--	--	--	7	10	168	130
OR26	15	14	145	70	20	10	187	107
OR27	15	31	150	93	20	23	212	122
OT06	15	8	117	51	--	--	--	--
OT08	15	30	205	98	25	40	164	102
OT09	15	23	224	93	20	11	149	116
OT10	15	22	171	90	20	32	177	112
OT12	15	10	167	73	11	23	135	115
OR15	15	33	215	88	--	--	--	--
OR22	32	6	150	78.5	--	--	--	--
OT07	10	13	208	89	--	--	--	--

Appendix Table 4. Female Wood Frog (*Lithobates sylvatica*) body measurements of snout-vent length (SVL) and mass for 8 sites in Bangor, Hampden, Orono, and Old Town, Maine, USA, in April 2016; median and range of observed values (in parentheses).

Site	n	SVL (mm)	Mass (g)
B09	6	52.5 (46–54)	18.5 (14–22)
B12	1	47	14
B23	2	59 (56–62)	24.5 (22–27)
H01	1	52	20
OR16	2	57 (55–59)	24.5 (21–28)
OR21	1	54	21
OT06	1	53	18

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