

## ARTICLE

## Freshwater Ecology

Assortative mixing in eastern spadefoot (*Scaphiopus holbrookii*) spatial networks is driven by landscape featuresAnne Devan-Song<sup>1,2</sup>  | M. A. Walden<sup>3</sup>  | James R. Watson<sup>4</sup> | Anna E. Jolles<sup>1,5</sup> | Justine M. Fox<sup>2</sup> | Nancy E. Karraker<sup>2</sup><sup>1</sup>Department of Integrative Biology, Oregon State University, Corvallis, Oregon, USA<sup>2</sup>Department of Natural Resources Science, University of Rhode Island, Kingston, Rhode Island, USA<sup>3</sup>Department of Natural Resources and Environmental Science, University of Nevada, Reno, Reno, Nevada, USA<sup>4</sup>College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, Oregon, USA<sup>5</sup>Carlson College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, USA

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## Abstract

Biotic and abiotic factors drive assortative mixing (preference for or sorting with individuals with similar characteristics) in animal populations on a landscape, with implications for dispersal, population structuring, and other ecological and evolutionary processes. However, patterns and generative mechanisms of assortative mixing are overlooked in amphibians outside of specific life history events such as reproduction. The aims of this project were to determine whether there is assortative mixing by size and life history category in eastern spadefoots (*Scaphiopus holbrookii*), whether these patterns are preserved across time and spatial scale, and quantify the nature and relative role of various habitat and soil features in explaining observed patterns in spatial organization of individuals. We conducted field surveys in southeastern Virginia, USA, in 2016 and 2017 during nonbreeding periods to create spatial networks of *S. holbrookii*. We quantified spatial assortativity by size and life history stage and evaluated the roles of multiple landscape features in explaining spatial organization of *S. holbrookii*. We found that *S. holbrookii* sorted spatially by size and sex outside of breeding periods, with juveniles and adults less likely to sort with each other. Within each life history stage, *S. holbrookii* sorted by size. These patterns were similar across time and spatial scale. Soil and habitat types had no effect on assortativity. Instead, the distance to nearest breeding pool, wetland, and meadow were related to life history stage assortativity, as well as size assortativity in males and subadults. Adult males and females displayed affinity for breeding pools and meadows and avoidance of other types of wetlands, while subadults and nonbreeding adults showed opposite patterns compared with breeding adults. Our results indicate that (1) previously established guidelines for the minimum size of buffer zones to protect wetland-breeding amphibians may be inadequate, (2) nonbreeding wetlands may be important core habitat for subadults, and (3) the upland spatial organization of amphibians may be used to predict locations of undetected breeding pools.

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**KEYWORDS**

breeding pools, extreme gradient boosting, life history, Monte Carlo methods, network analysis, Scaphiopodidae, spatial analysis, vernal pool

**INTRODUCTION**

Animals aggregate and sort in nonrandom patterns on the landscape due to biotic and abiotic factors. One of many resulting nonrandom patterns arising from heterogeneous spatial positions within a group can manifest and be quantified as assortative mixing (Newman, 2002; Peel et al., 2018), where similar individuals are more likely to associate or sort within proximity of each other. Assortative (or the inverse, disassortative) mixing can be observed across animal taxa including sex and age assortativity in bats (Zeus et al., 2018), cooperative phenotype in fish (Brask et al., 2019), and even through gentrification in human neighborhoods (Butcher & Dickens, 2016). While assortative mixing in animals is often behaviorally driven (Boyland et al., 2016; Lusseau et al., 2006; Mielke et al., 2020), the role of resource distribution in shaping assortative mixing patterns has also revealed interesting insights. Resource arrangement dictates how animals, including humans (Watson et al., 2018), use space (Heupel et al., 2019; Ullmann et al., 2018). These patterns can result in heterogeneity in intraspecific spatial proximity (Bachorec et al., 2020; Weigl, 1978). Spatial assortative mixing has ecological and evolutionary consequences for processes that shape animal populations, including collective movement (Briard et al., 2021), predator avoidance (Klamser & Romanczuk, 2021), disease transmission (Cobey et al., 2017; Perkins et al., 2008; Vos et al., 2018), and phenotypic variation and fitness at various scales (Berthouly-Salazar et al., 2012; Lee, 2011; Lowe et al., 2015). However, assortative mixing in animal populations has mostly focused on species known to be gregarious, overlooking taxa perceived to be more solitary and nonsocial.

Like most other ecological or evolutionary processes in amphibians, aggregation and intraspecific spatial patterns are mostly studied within the context of adult breeding and metamorph dispersal (Cayuela et al., 2020; Semlitsch, 2008). Few studies examine the role of landscape in shaping the nonbreeding spatial arrangement of amphibians, even though the biphasic lifestyle of many species would suggest tight linkages between aquatic resources and terrestrial spatial patterns with conspecifics (although see Kruger et al., 2021). Outside of breeding events, amphibians are generally considered to be solitary, and research into their upland (i.e., non-wetland) life history, intraspecific interactions, and ecology is

sparse. Focusing solely on specific life history events such as breeding can introduce bias into metrics of assortative mixing (Rios Moura et al., 2021). This research gap also signals the lack of information available to examine assortative patterns within an amphibian's entire life, the vast majority of which is not spent mating or dispersing.

Eastern spadefoots (*Scaphiopus holbrookii*) are a useful study organism to examine the role of habitat on amphibian spatial arrangement and assortative mixing on the landscape outside of breeding or dispersal aggregations. Ranging throughout much of the eastern United States (Conant & Collins, 1998), these fossorial anurans display a xeric or explosive breeding strategy (Gosner & Black, 1955; Wells, 1977). Adults migrate from the vicinity of their solitary, self-dug burrows in forests to form breeding aggregates in ephemeral pools with extremely short hydroperiods (<1 month) filled by torrential rains (hereafter termed "breeding pools"), where males begin calling to attract females (Bragg, 1945; Neill, 1957; Palis, 2012). Rainfall amounts necessary to attract eastern spadefoots to breeding pools do not occur every year, and populations are known to skip breeding in drier years (Gosner & Black, 1955). Hatching, development to metamorphosis, and metamorph dispersal away from breeding pools occur in a remarkably short time, sometimes in as little as 2 weeks, before breeding pools dry up (Palis, 2012). For the rest of the year on nonbreeding nights, adults emerge regularly from their burrows to forage in forests (Devan-Song et al., 2021), traveling short distances between several burrows (Pearson, 1957; Timm et al., 2014). Although soil types determine where spadefoots are able to dig burrows (Baughman & Todd, 2007; Moran & Button, 2011), their life history is tightly linked to the extreme unpredictability and ephemerality of breeding pools. The forests and wetlands used by *S. holbrookii* provide a useful study system for understanding how habitat features shape assortative mixing in amphibians, especially highly terrestrial species.

The aims of this project were to (1) determine whether there is size-and-stage assortative mixing in the spatial proximity of *S. holbrookii* and at what scale this may occur, (2) determine whether spatial patterns are temporally preserved, (3) quantify the relative contribution of various habitat features in explaining observed patterns in the spatial organization of individuals, and (4) describe and characterize the nature and spatial scale of habitat influence on assortative mixing. This investigation will aid our understanding of nonbreeding spatial

patterns of eastern spadefoots and identify possible mechanisms for spatial assortative mixing of amphibians on the landscape.

## METHODS

### Study site and landscape features

The workflow for this study is summarized in Figure 1. We conducted field surveys in the Yorktown Battlefield Unit of Colonial National Historical Park (37.216, -76.511, 0–21 m elevation), located in York County in the coastal plain physiographic region of southeastern Virginia, USA (Figure 2a,b). Meadows are mowed in this unit to preserve the historical and cultural features of the battlefield, while meadow vegetation bordering roads are mowed even more frequently. This vegetation

maintenance creates ideal environments for small, flooded puddle-like pools in open vegetation (i.e., short grasses and forbs) near roads that are adjacent to meadows. These small pools are used for mating and egg deposition by eastern spadefoots almost exclusively. That is, few amphibian species successfully use these pools for breeding, and *S. holbrookii* mostly does not use other pools with longer hydroperiods for breeding.

Eastern spadefoot choruses of calling males can be heard from many hundreds of meters away from breeding pools, and we documented the location of 11 breeding pools incidentally during mass breeding events in 2015. We obtained one more breeding wetland location from 2008 surveys conducted at Yorktown Battlefield (T. Christensen, personal communication). All known breeding pools were either within or immediately adjacent to meadows, particularly in areas with short vegetation maintained by mowing and landscaping throughout the park. We obtained spatial

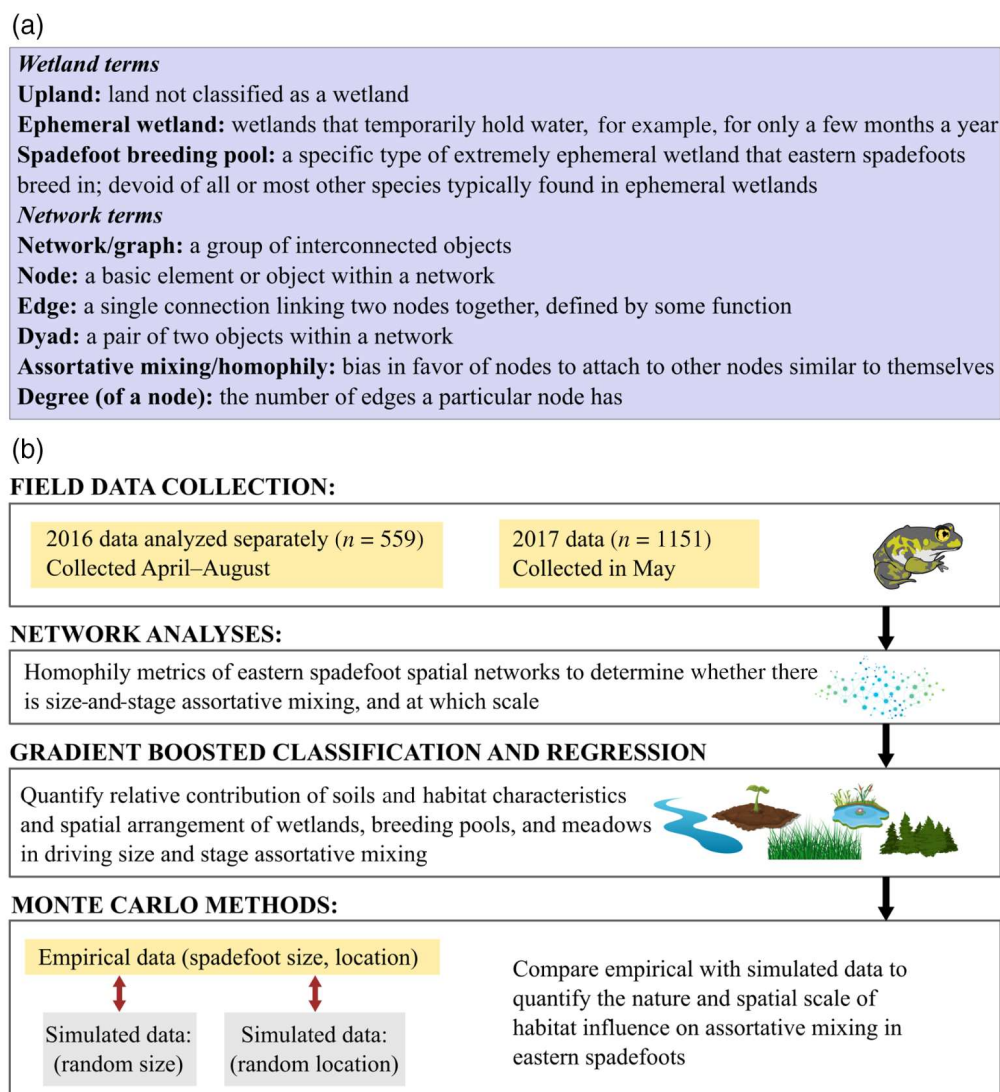


**FIGURE 1** Glossary of terms (a) and workflow for this study (b). Empirical data for this project are highlighted in yellow, while simulated data are in gray.

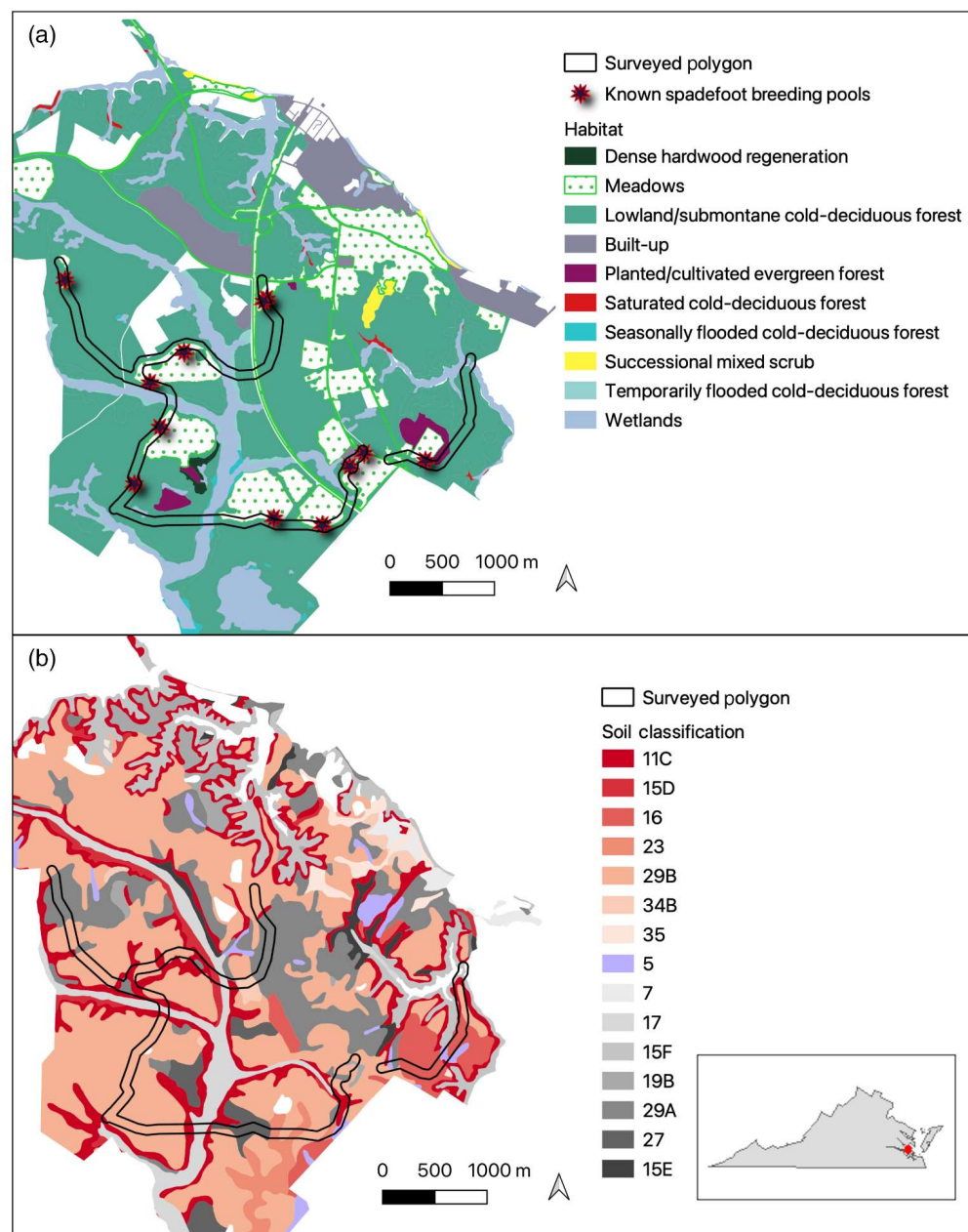


**FIGURE 2** Habitat type (a) and soil classification (b) of survey area in Yorktown Battlefield Unit, Colonial National Historical Park, Virginia, USA. Soil legend: 5 = Bethera silt loam, 7 = Bojac sandy loam, 11C = Craven–Uchee complex 6%–10% slopes, 15D = Emporia complex 10%–15% slopes, 15E = Emporia complex 15%–25% slopes, 15F = Emporia complex 25%–50% slopes, 16 = Izagora loam, 17 = Johnston complex, 19B = Kempsville–Emporia fine sandy loams 2%–6% slope, 23 = Newflat silt loam, 27 = Peawick silt loam, 29A = Slagle fine sandy loam 0%–2% slopes, 29B = Slagle fine sandy loam 2%–6% slopes, 34B = Uchee loamy fine sand 2%–6% slopes, 35 = Udorthents loamy.

data layers for 10 habitat features within the study site that encapsulate the variation of landscape features at the Yorktown Battlefield Unit (Table 1, Figure 2a,b; Appendices S1 and S2) and hypothesized that it may affect eastern spadefoot distribution. Spatial data layers were obtained from Virginia Department of Conservation and Recreation (2008) and National Park Service Inventory and Monitoring Program Soil Resource Inventory (2012). These data layers included characteristics and texture of soils, habitat type and vegetation

alliance, and locations of the major distinct features in the unit (meadows and wetlands; Figure 2a). The category “all other wetlands” included perennial and ephemeral wetlands that were not breeding pools used by eastern spadefoots. Eastern spadefoot breeding pools are a unique subset of ephemeral pools with extremely short hydroperiods (~1 month a year), do not support most of the plant and animal indicators of ephemeral wetlands, and are generally much smaller than the average ephemeral wetland.

**TABLE 1** Landscape features, data sources, and data extraction methods used to predict stage and size of eastern spadefoots (*Scaphiopus holbrookii*) in the Yorktown Battlefield Unit of Colonial National Historical Park, Yorktown, Virginia, USA.

| Data source                                                   | Landscape feature covariates                                                                  | Methods                                                                 |
|---------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| 2015 surveys                                                  |                                                                                               |                                                                         |
|                                                               | Spadefoot breeding ponds                                                                      | Calculated distance of each data point to nearest breeding pool         |
| Virginia Department of Conservation and Recreation (2008)     |                                                                                               |                                                                         |
|                                                               | All other wetlands                                                                            | Calculated distance of each data point to nearest non-spadefoot wetland |
|                                                               | Meadows                                                                                       | Calculated distance of each data point to nearest meadow                |
|                                                               | Habitat: formal name and vegetation alliance                                                  | Extracted by overlaying spadefoot locations with habitat type           |
| National Park Service Inventory and Monitoring Program (2012) |                                                                                               |                                                                         |
|                                                               | Soil classification: top and middle layer texture, drainage, parent material, and hydric soil | Extracted by overlaying spadefoot locations with soil type              |

For the duration of this study, all eastern spadefoot breeding pools were dried up, and no breeding- or dispersal-related migration took place.

Field data collection

Surveys were conducted from April to August in 2016 and 1 week in May 2017 for this study. All known eastern spadefoot breeding pools were dry for the duration of this study, and daily precipitation was minimal (Appendix S3). All surveys started at least half an hour after sunset. For the 2017 surveys, two surveyors were assigned either the left or right edge of roads within Yorktown Battlefield and surveyed the forest floor adjacent to the road along non-overlapping sections of the 16-km tour road. The 2016 surveys were conducted similarly but did not include certain sections of the road (compare Figure 3a,b). Surveyors located eastern spadefoots using spotlighting for eyeshine following the methods described in Devan-Song et al. (2021). The relatively flat and open landscape of this site allowed for the detection of eyeshines

up to nearly 50 m from a surveyor (Devan-Song et al., 2021). When we spotlighted an eastern spadefoot, we left the road, captured the individual for processing, and recorded GPS location with a handheld unit ( $\pm 5$  m). We measured snout-vent length with digital calipers ( $\pm 0.01$  mm) and recorded the presence of nuptial pads on front toes, or eggs which can be seen through the translucent inguinal skin in this species. Individuals with eggs were recorded as mature females (F), individuals with nuptial pads were recorded as mature males (M), and all other individuals were recorded as subadults/nonbreeding adults (SNBA) as we could not determine sex in the field without secondary sexual characteristics. We further subdivided SNBA into nonbreeding adults (NBAs) if they were above 44 mm in snout-vent length, and subadults (S) if they were below 44 mm in snout-vent length, using average size at sexual maturity (Conant & Collins, 1998) as a threshold. The combination of life history and sex categories (female, male, NBA, and subadult) are hereafter referred to as “stages.” We released individuals at the site of capture and resumed the surveys. We did not capture individuals that were not detected directly from the edge of the road. Using an individual’s GPS coordinates, we extracted 10 landscape attributes or features from the spatial data layers associated with each capture (Table 1).

Network creation and metrics

We used the igraph package (Csardi & Nepusz, 2006) to construct networks from the frog proximity data and calculate network and node metrics. We defined our eastern spadefoot network as individual frogs, each represented as a *node* and occupying home ranges with a diameter of 25 m. We chose 25 m as a conservative estimate of extent of home range diameter, which excluded breeding migration, based on a study that quantified upland movements of eastern spadefoots (Timm et al., 2014). If two frogs were detected within 25 m of each other, we defined them as a *dyad* (two connected individuals within the network) with an undirected and unweighted *edge* (connection between the dyad). Node-level attributes included stage (female, male, NBA, and subadult) and size (snout-vent length in millimeter).

We extracted the *degree* (number of frogs an individual frog was connected to) distribution from the network by stage and used an Anderson–Darling test of equal distributions to determine whether the degree distributions for the four stages differed from each other. We calculated the assortativity coefficient ( $r$ ; between  $-1$  and  $1$ ) to determine whether there was a tendency for individuals within the same stage to associate with each other (Newman, 2002). We also calculated the assortativity coefficient by

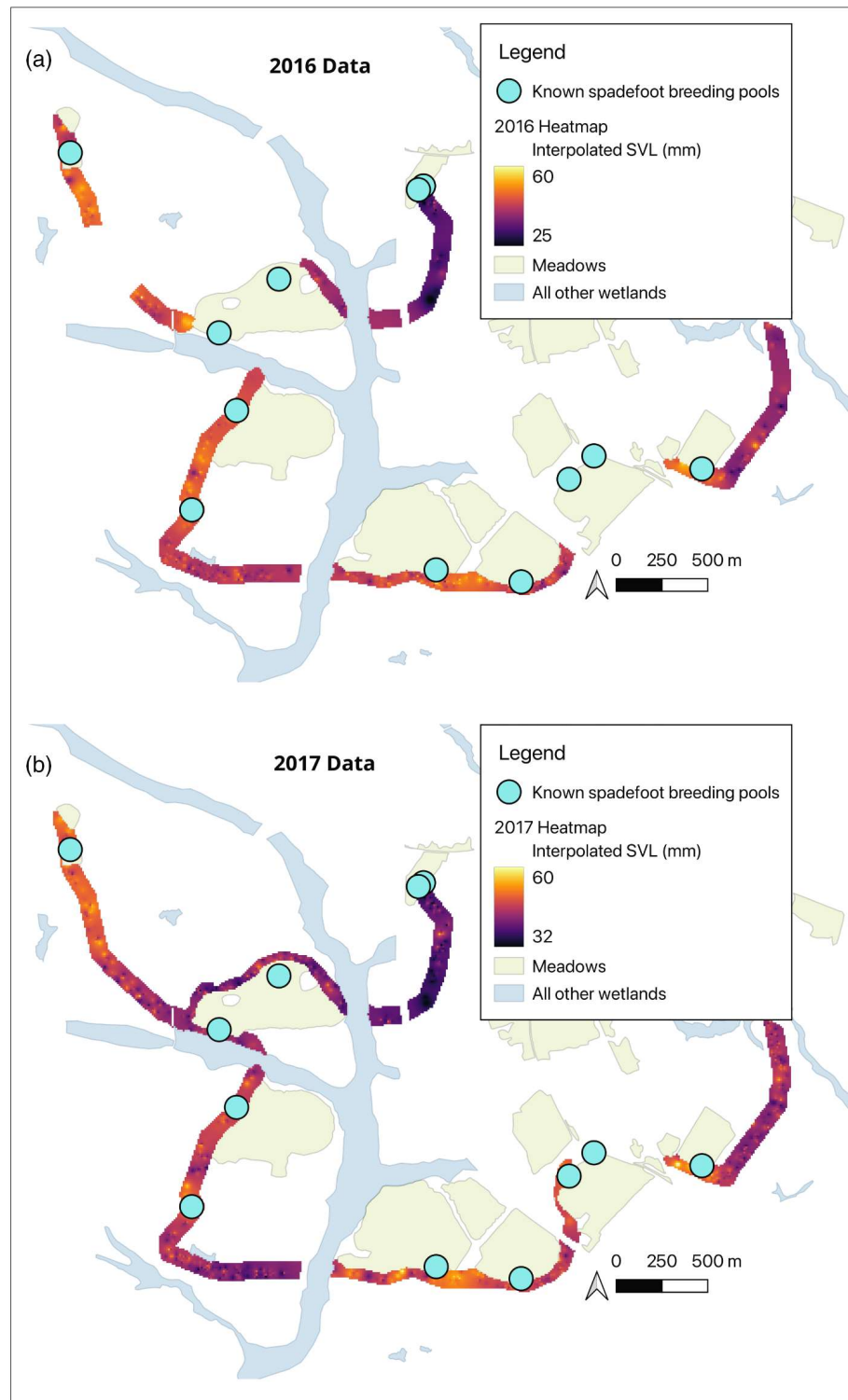


**FIGURE 3** Interpolated heatmap of eastern spadefoot (*Scaphiopus holbrookii*) snout-vent length (SVL) across the study polygon, using GPS coordinates of each individual as  $x, y$  points and SVL as interpolated  $z$ -coordinates across  $10 \times 10$  m pixels within the study polygon in study years 2016 (a) and 2017 (b). Data collected from Yorktown Battlefield Unit of Colonial National Historical Park, Yorktown, Virginia, USA.

snout-vent length across the entire network to determine whether there was a tendency for similar-sized individuals to associate with each other. Finally, we subdivided the networks into stages and calculated the within-stage snout-vent length assortativity. An assortativity coefficient of

$r > 0$  indicates a tendency for nodes to associate with similar nodes, while  $r < 0$  indicates the tendency for nodes to associate with dissimilar nodes. To obtain standard error for all categorical and numerical estimates of assortativity, we used jackknifing with the following equation:

$$\sigma_r^2 = \sum_{i=1}^M (r_i - r)^2, \quad (1)$$

where  $r_i$  is the value of assortativity  $r$  for the network in which the  $i$ th edge is removed, and  $M$  is the number of edges in the network. From the sigma values, we calculated 95% CI for all the above estimates of assortativity using  $z = 1.96$  as the critical value for a two-tailed test following Newman (2002).

We performed a  $\chi^2$  test of the proportion of realized edges between nodes by stage, using the proportion of individuals in different stages as expected values, and visualized the residuals in a correlation plot. That is, the null hypothesis predicts that any particular frog's connections to frogs of each stage are purely a result of sample sizes within stages, not a preferential attachment.

All the network analyses were conducted on both the 2016 and 2017 data to determine whether there was temporal preservation of assortative mixing patterns. Finally, we repeated the analyses on the 2017 data using different thresholds for edge definition in order to compare assortativity patterns when edges are defined by 10 and 100 m instead of the 25 m threshold as described above.

## Relative importance of landscape features in predicting stage and size

We used extreme gradient boosting in the XGBoost package (Chen et al., 2015) to quantify the relative importance of landscape features in predicting an eastern spadefoot's stage and size based on its location. Extreme gradient boosting is a tree-based ensemble approach to classification and regression that prevents overfitting of models through regularization (Chen & Guestrin, 2016) and calculates the relative importance of each covariate of interest in correctly predicting the dependent variable. For stage prediction, we used multiclass classification to calculate the relative importance of 10 landscape variables (Table 1) in accurately predicting a frog's stage (female, male, NBA, and subadult) based on its GPS coordinates. For size prediction, we used gradient boosted regression to calculate the relative importance of these same 10 landscape variables (Table 1) in accurately predicting a frog's snout-vent length based on its GPS coordinates.

## Quantifying relationships between habitat features and stage assortativity

We created a spatial polygon dataset representing the area surveyed in the Yorktown Battlefield by extracting a 50-m buffer around the surveyed roads. We chose a 50-m

buffer based on our spotlighting efficacy in this habitat (see Devan-Song et al., 2021), where the topography and habitat type allow for the detection of frogs far away from the surveyor. We further subsetted the survey polygon by removing areas of the polygon where an eastern spadefoot would not physically be able to burrow or forage (i.e., inundated perennial wetlands or streams).

As distances to the nearest breeding pool, meadow, and any other wetland were significant predictors of an eastern spadefoot stage (Figure 4), we conducted the following analyses for these three landscape features. We compared our empirical data with simulations to determine whether eastern spadefoots were nearer a landscape feature than expected at random. These simulations were conducted by subsetting the data by stage (female, male, NBA, and subadult) and then completing the following analyses for each stage. We determined the farthest Euclidean distance an individual frog was found from all three landscape features of interest, which was 879 m. We calculated the Euclidean distance of each individual to the nearest feature. This allowed us to calculate the empirical distribution of the number of eastern spadefoots within distance  $j$  of any landscape feature, where  $j$  represents 1-m increments from 1 to 879 m away from the landscape feature. Next, we generated random points ( $X$ ,  $Y$  coordinates) within the subsetting polygon matching the sample sizes of each stage and calculated the Euclidean distance of each random point to the nearest landscape feature. We obtained the empirical distribution of these random points within distance  $j$  of any landscape feature, where  $j$  represents 1-m increments from 1 to 879 m away from the landscape feature. We repeated this simulation 1000 times and calculated the proportion of simulated datasets at every meter away from a landscape feature that were larger or smaller than empirical data values. That is, at every distance  $j$  from 1 to 879 m, we calculated the proportion of the 1000 simulated data values that were equal to or smaller than the empirical data values using the following formula:

$$I = \begin{cases} 1, & N_j - X_{jm} \leq 0 \\ 0, & N_j - X_{jm} > 0 \end{cases}, \quad (2)$$

$$P_j = \left( \sum_{m=1}^k I \right) / k$$

where  $N_j$  is the number of frogs within  $j$  meters of a landscape feature, and  $X_{jm}$  is the number of random points within  $j$  meters of a landscape feature at the  $m$ th simulation. We considered any values of  $j$  as having a significantly different number of frogs within 0- $j$  m of the landscape feature than expected at random only if  $P_j \geq 0.9958$  or  $P_j \leq 0.0042$ . An alpha ( $\alpha$ ) error of 0.0083 was chosen by the Bonferroni correction, dividing 0.05/6 as we used six different Monte Carlo analyses in this study.

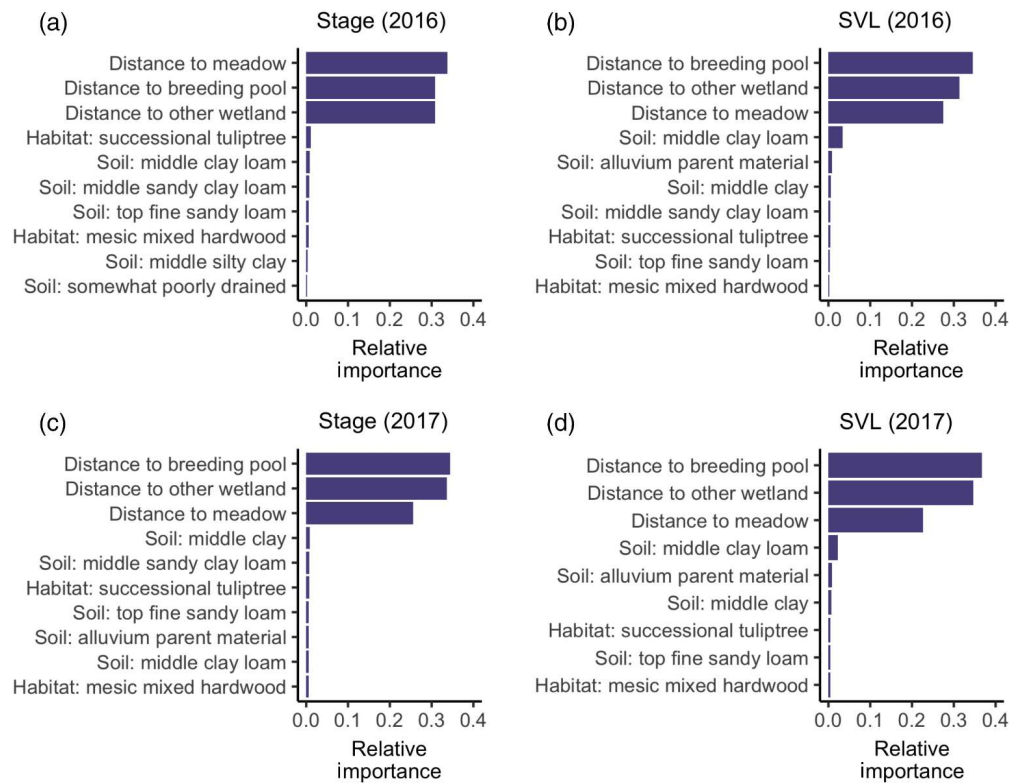


**FIGURE 4** Relative importance of each landscape feature in predicting stage and snout-vent length of eastern spadefoots (*Scaphiopus holbrookii*) using extreme gradient boosted multi-class classification and regression for 2016 (a, b) and 2017 (c, d) at Yorktown Unit, Colonial National Historical Park, Virginia, USA.

### Quantifying relationships between habitat features and body size assortativity

We performed 1000 Monte Carlo simulations to determine whether average frog size nearer the landscape feature (breeding pool, meadow, and any other wetland) were significantly different than expected at random. We subset the data by stage (female, male, NBA, and subadult) and completed the following analyses for each category. We obtained the empirical distribution of the snout-vent lengths of all frogs within distance  $j$  of any landscape feature, where  $j$  represents 1-m increments from 1 to 879 m. We then assigned a snout-vent length to the (X, Y) location for each individual, drawn with replacement from the empirical distribution of snout-vent lengths in order to randomize the size allocated to each point. We calculated the sum of snout-vent length of all frogs within  $j$  meters of the nearest landscape feature for each simulated dataset. At every distance  $j$ , we then calculated the proportion of simulated size summations that were equal to or smaller than the empirical size summations using the following formula:

$$I = \begin{cases} 1, & C_j - S_{jm} \leq 0 \\ 0, & C_j - S_{jm} > 0 \end{cases}, \quad (3)$$

$$Q_j = \left( \sum_{m=1}^k I \right) / k$$

where  $C_j$  is the sum of snout-vent lengths in millimeters of all frogs within  $j$  meters of a landscape feature, and  $S_{jm}$  is the sum of randomly assigned snout-vent lengths for all frog points within  $j$  meters of a landscape feature at the  $m$ th simulation. Using a significance value of alpha ( $\alpha$ ) of 0.0083, we considered any distances of  $j$  as having significantly different sized individuals within 1 to  $j$  meters of a landscape feature than expected at random only if  $Q_j \geq 0.9958$  or  $Q_j \leq 0.0042$ . All data analyses for this study were conducted in Python 3.7 (Van Rossum & Drake, 2009), R v3.6.2 (R Core Team, 2021), and QGIS (QGIS.org, 2022).

### RESULTS

After removing data points that had missing snout-vent length values and/or stage (30 individuals), our dataset consisted of 559 individuals in 2016 (93 females, 125 males, 76 NBAs, and 265 subadults) and 1151 individuals in 2017 (243 females, 299 males, 74 NBAs, and 535 subadults). Visual inspection of heatmaps created through interpolation of snout-vent length across survey polygons showed similar patterns in 2016 and 2017 (Figure 3).

## Comparing network analysis results between years and edge definitions

Degree distributions for the different eastern spadefoot stages were different in the 2016 network (T.AD = 3.041,  $p = 0.013$ ; Figure 5a). This same pattern was observed in the 2017 network (T.AD = 4.867,  $p = 0.001$ ; Figure 5d). In both years, we found significant positive assortativity for all groups of interest except for size assortativity within the NBA stage, which was assorted by size in 2016 but very weakly disassortative by size in 2017 (Figure 5b,e). Female and male *S. holbrookii* were more likely to associate within their stages (Figure 5c,f). Females

and males were less likely to associate with subadults (and vice versa), and subadults were more likely to associate with other subadults (Figure 5c,f). Patterns for NBAs, whether avoidance or affinity, were relatively weak (Figure 5c,f). These same patterns were observed both in 2016 ( $\chi^2 = 268.19$ ,  $df = 9$ ,  $p \ll 0.001$ ; Figure 5c) and 2017 ( $\chi^2 = 555.91$ ,  $df = 9$ ,  $p \ll 0.001$ ; Figure 5f). Besides size assortativity within the NBA stage, all assortativity patterns were similar between 2016 and 2017 (Figure 5b,e).

Regardless of the definitions of network edges (10, 25, or 100 m), the same general patterns were observed in the 2017 networks (Figure 5; Appendix S4), with the exception of females. Females showed disassortative

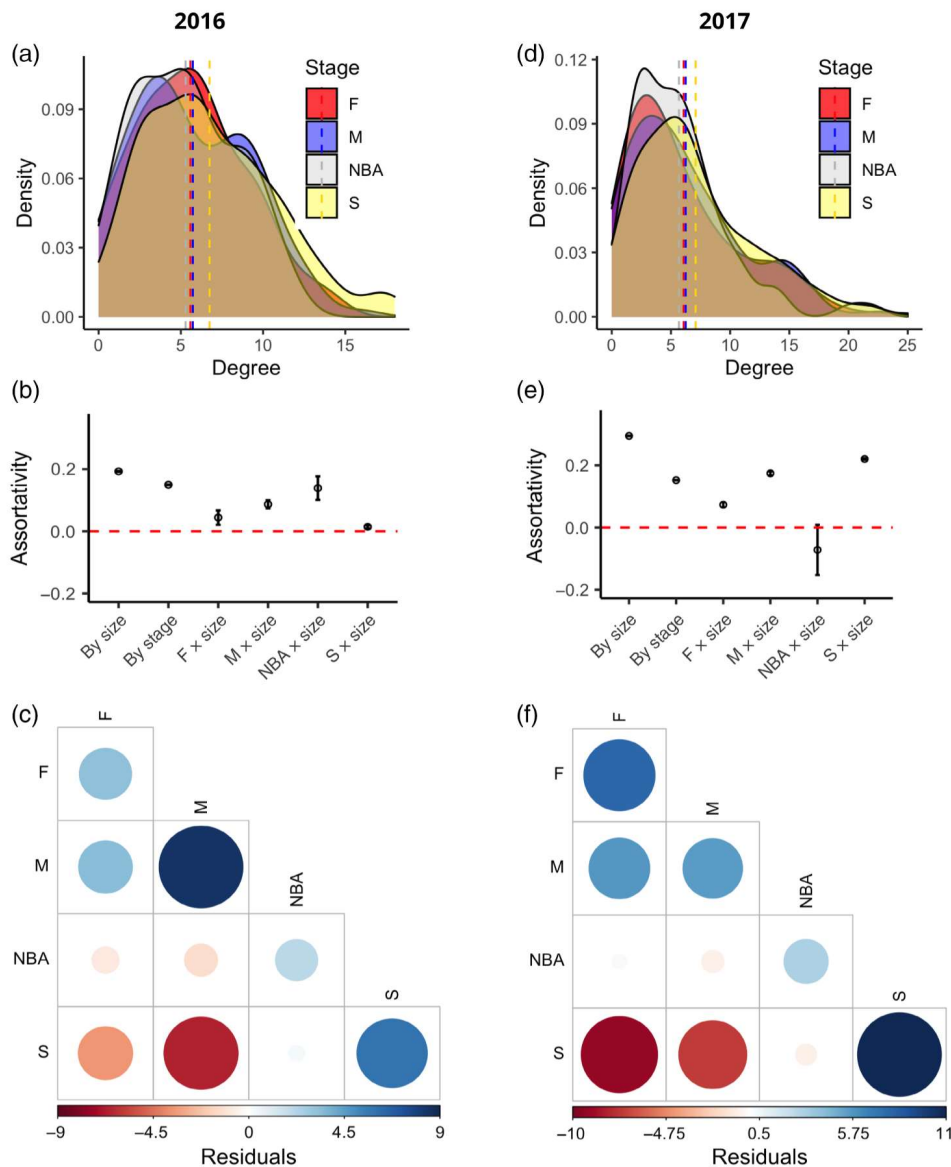


**FIGURE 5** Assortative mixing metrics for eastern spadefoots (*Scaphiopus holbrookii*) in 2016 (a–c) and 2017 (d–f) from networks with edges defined by 25 m thresholds. Degree distributions of different stages (a, d), network assortativity by various categories (b, e), and correlation plot of  $\chi^2$  test residuals for the proportion of homogenous and heterogenous dyads by stage (c, f). Circle sizes correlate with magnitude of residuals. F = female, M = male, NBA = nonbreeding adult, and S = subadult. Data collected from Yorktown Battlefield Unit of Colonial National Historical Park, Yorktown, Virginia, USA.

mixing when network edges were defined by 10-m thresholds and assortative mixing when network edges were defined by 25-m thresholds and 100-m thresholds (Figure 5; Appendix S4). The same general patterns of avoidance and affinity between stages were observed for all network definitions although the strengths of affinity and avoidance were highest in the network defined by 100-m edge threshold (Figure 5; Appendix S4). Degree distributions between stages were consistently different from each other regardless of network edge definition, with subadults displaying higher average degree number per frog than other stages (Figure 5; Appendix S4).

### Relative importance of landscape features in predicting stage and size

Regardless of year or which variable of interest we attempted to predict (i.e., size or stage), the relationships between landscape features and frog attributes were consistent. A frog's distance to the (1) nearest breeding pool, (2) nearest meadow, and (3) nearest other wetland were far more important in predicting its stage and size than all other landscape features (Figure 4). Soil and habitat characteristics had negligible importance in predicting a frog's stage and size, given its location. For 2017, the distance to nearest breeding pool appeared to have better predictive power of a frog's stage and size than the distance to the nearest meadow or other wetland (Figure 4c,d).

### Relationships between habitat features and stage assortativity

Eastern spadefoots showed strong affinity for or avoidance of breeding pools depending on their stage. More females and males were found nearer to breeding pools than expected at random (Figure 6a,b), while more NBAs and subadults were found farther away from the pools than expected at random (Figure 6c,d). However, these patterns were only detectable in NBAs at distances far away from breeding pools (Figure 6c), unlike other stages that displayed strong signals of affinity or avoidance nearer the breeding pools (Figure 6a,b,d).

Eastern spadefoots showed some affinity for or avoidance of meadows depending on their stage. Like distribution around breeding pools, female and male eastern spadefoots showed some affinity for meadows (Figure 6e,f), while NBAs and subadults displayed avoidance of meadows (Figure 6g,h). However, the patterns were not as strong for females and males with relationships to meadows, when compared to their relationships to breeding pools (Figure 6a,b,e,f).

Eastern spadefoots showed strong affinity for or avoidance of other types of wetlands depending on their stage; however, these patterns were opposite from what was observed in relation to breeding pools and meadows. Adult females and males avoided other types of wetlands (Figure 6i,j) while NBAs and subadults showed affinity for other wetlands (Figure 6k,l), with a very strong signal for subadults (Figure 6l).

Overall, adult female and male eastern spadefoots were more likely to be nearer breeding pools and meadows (Figure 6a,b,e,f), and further away from other wetlands (Figure 6i,j), than randomly generated datasets. NBA and subadult eastern spadefoots were more likely to be nearer than other wetlands, and farther away from breeding pools and meadows, than randomly generated datasets. The spatial scale of this relationship was larger for breeding pools and other wetlands than for meadows, where the range of significant distances were smaller in meadows (blue- and pink-shaded areas; Figure 6a-l).

### Relationships between habitat features and body size assortativity

Only two stages showed any significant relationships between body size and distance to features. Male frogs within a small range of distances (84–158 m) near breeding pools were larger in size than expected (Appendix S5). Subadult frogs within a range of distances (52–240 m) near other wetlands were larger in size than expected (Appendix S5).

## DISCUSSION

Our study shows that eastern spadefoots display nonrandom spatial proximity patterns within populations: Individuals assort spatially by body size and stage, and adults have fewer spatial network connections to conspecifics than subadults. These patterns were observed in both study years. For all stages but adult females, assortativity patterns were similar regardless of the edge definitions of spatial networks. Depending on the stage of a spadefoot, we detected either strong avoidance of or affinity for habitat features (breeding pools, meadows, and other wetlands). However, habitat features only weakly explained size assortativity. Understanding the spatial patterns of eastern spadefoots has theoretical relevance for the study of amphibian spatial ecology, but also several implications for the conservation of wetlands and ephemeral wetland-breeding amphibian populations.

The preservation of patterns across spatial scales in both study years indicates a strong signal of assortativity by stage and body size of eastern spadefoots on the landscape, rather

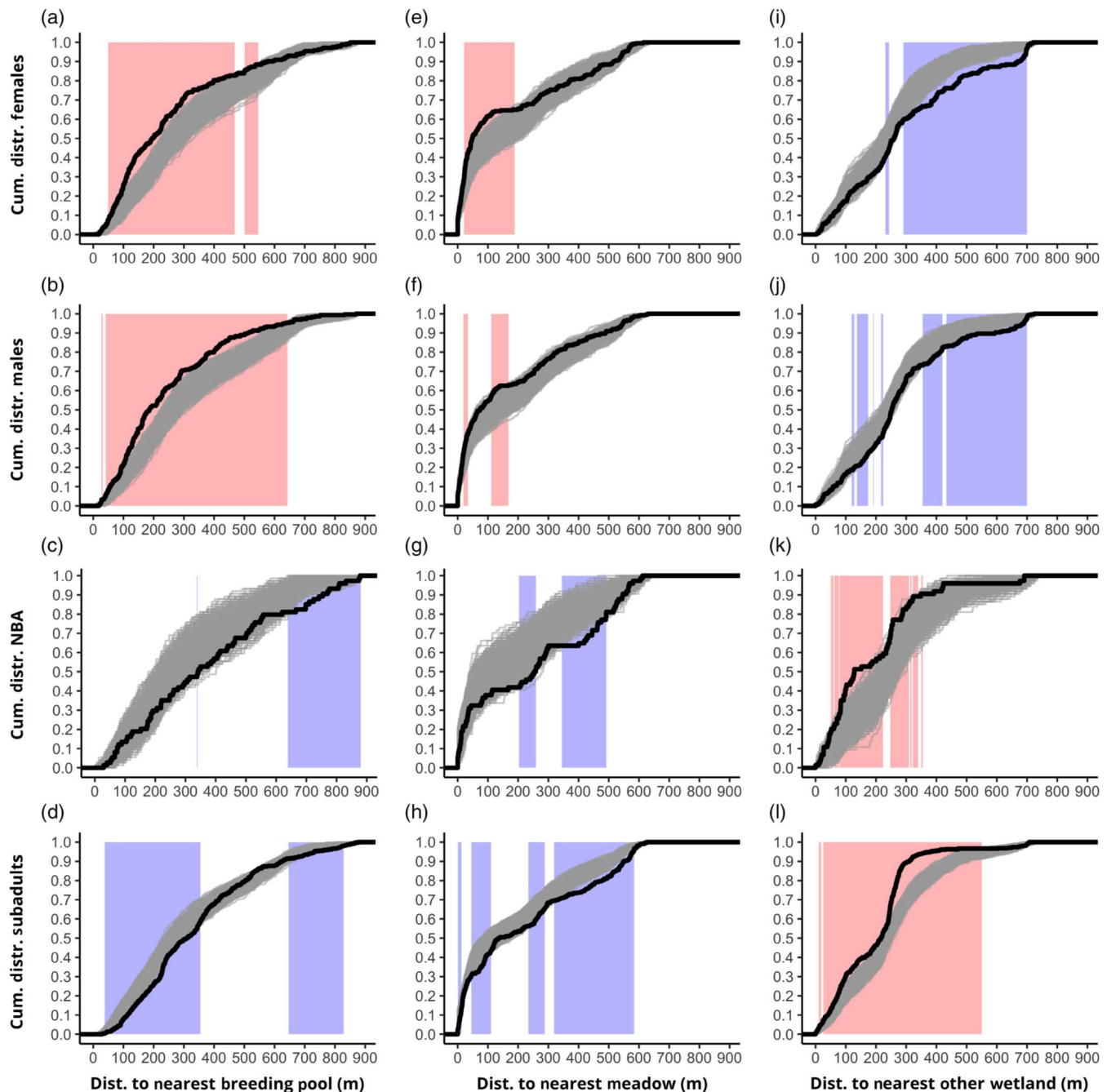


**FIGURE 6** Empirical cumulative distribution functions of number of eastern spadefoot (*Scaphiopus holbrookii*) at each distance from landscape features (black line) and cumulative distribution functions of number of simulated points (gray lines) for 1000 simulations for each stage (female, male, nonbreeding adult [NBA], and subadult). X-axis values for which the empirical data are significantly different ( $\alpha = 0.0083$ ) from simulated data are highlighted in pink if there is affinity for a feature, and purple if there is avoidance of a feature. Data collected from Yorktown Battlefield Unit of Colonial National Historical Park, Yorktown, Virginia, USA.

than being an artifact of edge definitions within spatial networks or a function of survey dates. The only exception was females, which showed disassortative size patterns at smaller scales (10 m edges). Female size assortativity was also consistently lower than males, suggesting that size assortativity in females is comparatively weak in general. The other exception involved NBAs, which show opposite patterns of size assortativity patterns across years. However,

sample sizes for NBAs were relatively small in both years (76 in 2016 and 74 in 2017), and the same general patterns in avoidance of and affinity for different stages were observed in both 2016 and 2017 NBAs (Figure 5c,f). No breeding events occurred at our site during 2016 and 2017, so we cannot make any conclusions about temporal preservation of these patterns pre- and post-breeding. Site fidelity has been observed in eastern spadefoots (Pearson, 1957),

however, suggesting that future studies documenting pre- and post-breeding spatial patterns may also find that these upland spatial patterns are consistent.

We note that none of the soil or habitat descriptors had any predictive power of an eastern spadefoot's stage or size, even though burrow site for this species is strongly dependent on soil characteristics (Moran & Button, 2011). Combined with our results, we suggest that soil and habitat characteristics predict occupancy (presence/absence) but not assortative mixing. Instead, distance to habitat features, which eastern spadefoots do not burrow in (wetlands and meadows), were a strong predictor of an individual's stage and size, given its location. These results indicate that avoidance of or affinity for certain features drives assortative mixing in eastern spadefoots. However, we did not investigate other possible explanatory variables of habitat features. Future studies may incorporate interspecies interactions such as prey and predator composition and availability, as well as environmental variables such as soil pH, humidity, and leaf litter depth.

Our results show that males and females display opposite patterns compared with subadults and NBAs with respect to habitat features. Males and females show affinity for dried-up breeding pools and meadows, and avoidance of other wetlands. We hypothesize that given the unpredictability of when breeding pools fill up with water, adult males and females may prefer to stay within proximity of dried-up pools in preparation for a sudden rain event. This relationship is flipped for subadults and NBAs: They show avoidance of breeding pools and meadows, and affinity for other wetlands. All the known spadefoot breeding pools in our study site are within or immediately adjacent to meadows, and relationships between spadefoots and breeding pools are stronger than for meadows. This finding suggests that female and male affinity for meadows is driven by the location of breeding pools rather than any characteristic of the meadows themselves. Additionally, we found that males and females avoid other wetlands, while juveniles and subadults have affinity for other wetlands. This pattern could be the result of smaller nonbreeding frogs being pushed to suboptimal locations nearer other wetlands that may have poorly drained soils or fewer terrestrial invertebrate prey which form most of adult spadefoot diets (Jamieson & Trauth, 1996; Whitaker et al., 1977). However, we cannot rule out actual affinity for other wetlands by nonbreeding individuals, as the diet and overall life history of eastern spadefoots are almost completely unknown for these life history stages (Conant & Collins, 1998).

We demonstrated that size assortativity among males and subadults can be explained partially by habitat features, and only within very narrow ranges of distances from these features. Larger males were found closer to dried-up breeding pools, which we hypothesize may have

implications for fitness. Male fitness in wetland-breeding anurans is correlated with the ability of males to attract mates in breeding wetlands (Doty & Welch, 2001), and time spent traveling to a wetland and obtaining a mate are important as the period male anurans spend calling is energetically costly (Gerhardt, 1994). We hypothesize that larger frogs may be able to defend burrows or territories nearer breeding pools, potentially excluding smaller males. Our results show that larger subadults were found closer to other wetlands (i.e., nonbreeding pools) than expected at random. This pattern may reflect differences in prey availability or composition at wetland edges, although we do not have prey data to explore this hypothesis. Neither of these patterns were particularly strong and occurred within a narrow band of distances to habitat features. The relationship between habitat features and size assortativity in males and subadults is weak, however, and other factors such as prey availability and composition or burrow selection may be more powerful drivers of these spatial patterns.

We found no evidence that habitat features influence assortativity by size among females or NBAs. Females and NBAs assort by size within their stage, but these clusters of similarly sized individuals appear to be unrelated to habitat features. We hypothesize that female fitness is determined more by clutch size (number of eggs laid) than distance to breeding wetland; therefore, foraging to increase body condition and clutch size is prioritized over proximity to wetland. This hypothesis is also supported by other research (Timm et al., 2014) that demonstrated that the largest adult female *S. holbrookii* in a population exhibited a home range 2.5 times larger than that of the largest male home range. Other potential explanations for within-stage size assortativity may be related to predator and/or prey distributions, or even behavioral preferences, which can drive assortativity in many animals (Mielke et al., 2020; Perkins et al., 2008; Vos et al., 2018) but are outside the scope of our data collection.

While breeding pools are themselves a tiny fraction of the habitat used by individuals, and are an infrequent and unpredictable resource, our results show that they are a strong driver of eastern spadefoot spatial structure on the landscape. Most importantly, we have shown that this spatial structure is maintained by the arrangement of ephemeral breeding wetlands even between resource pulses, when the wetlands have been drying for a year or more. Previous research has established that resources can drive facultative social interactions in amphibians (Cayuela et al., 2017; Pröhl et al., 2019), but stratification by life history category and body size for eastern spadefoots was detected in both study years even during the long periods of time when breeding pools remain dry. The relationship between ephemeral resources and nonrandom spatial arrangement is perhaps

unsurprising, given the significance of pulsed or patchy resources in driving inherently spatial processes such as movement (Robillard et al., 2016; Therrien et al., 2014), demography (Crone, 2013; Gamelon et al., 2017), and population dynamics (Russell & Ruffino, 2012).

In addition, our findings have three applications that relate to wetland conservation. Faunal-based wetland conservation usually includes protection of the inundated portion of a breeding pool, whether seasonal or permanent, and the surrounding upland area used by wetland associated species for all aspects of their nonbreeding life histories, including foraging, estivation, and seeking cover (Gibbons, 2003; Semlitsch & Bodie, 2003). Many wetland core habitat and buffer zone recommendations are derived primarily from information on adult amphibians (Semlitsch, 1998; Semlitsch & Bodie, 2003), although a study including subadult salamanders' distances to breeding sites suggests spatial stratification by life history stage (Searcy & Shaffer, 2008). Similarly, our results demonstrate upland spatial stratification by all life history categories. Wetland management policies that do not account for subadults and NBAs will underestimate buffer zones necessary for breeding pool conservation (Gamble et al., 2006).

Second, our results highlight subadult and NBA affinity for nonbreeding wetlands, which included both ephemeral and perennial wetlands that adult eastern spadefoots avoid and do not use for breeding. The majority of spadefoot field research focuses on breeding pools where adults, eggs, and larvae can be found (Devan-Song et al., 2021), overlooking the use of other types of wetlands by subadults and NBAs. Conservation efforts may be bolstered by including nonbreeding wetlands within the core habitat calculations for this species, and all life history stages should be included in these calculations.

Finally, our research may offer useful conservation-related tools, including the ability to locate short-hydroperiod ephemeral wetlands when they are dried up. Relying on active breeding events may be an impractical way to find eastern spadefoot breeding pools (Ryan et al., 2015), which can form almost any time of the year during torrential rains and have very short hydroperiods of a few weeks. The temporally preserved spatial structure of eastern spadefoots in uplands that we have demonstrated in this study may be useful in predicting the locations of breeding pools that have not yet been discovered. With site-specific samples of known breeding pool locations, it may be possible to build predictive models for locations of unknown breeding pools, which will be a highly valuable conservation tool for this species, particularly in parts of its range where ephemeral breeding sites are threatened by land use activities. For example, surveys identifying concentrations of large male frogs may

indicate the presence of a nearby dried-up pool, and auditory surveys for breeding choruses can be concentrated near these congregations in heavy rains.

This study further highlights the fact that the ecological footprint of an ephemeral breeding pool on the landscape is far greater than its size and hydroperiod would suggest. Ephemeral wetlands support distinct suites of plant and animal species, including vertebrate and invertebrate animals, many of which are declining or vulnerable and under threat from accelerating land development (Calhoun et al., 2017). The uncertainty and inconsistency surrounding regulatory protections of wetlands in the United States are only heightened for ephemeral wetlands (Waters et al., 2019). Further progress in understanding social structure of poorly understood taxa should lead to more effective protections for the generative processes fundamental to these spatial patterns in animals and hence lead to comprehensive guidelines better ensuring the persistence of at-risk species and communities into the future.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data and code (Devan-Song, 2022) are available from Zenodo: <https://doi.org/10.5281/zenodo.6575172>.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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