Multiscale Occupancy Patterns of Anurans in Prairie Wetlands

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ABSTRACT: Habitat loss and degradation appear to be the primary drivers of amphibian declines. Given the losses of native prairie and prairie wetlands, it is essential to understand the habitat conditions that support amphibian species in remaining prairie ecosystems. In this study, we combine wetland surveys, geographic information systems (GIS), and hierarchical statistical modeling to assess anuran occupancy relationships with wetland and landscape factors. We surveyed 141 wetlands with repeated sampling for amphibian breeding activity (calling, eggs, tadpoles, metamorphs) in the Sheyenne National Grasslands—one of the largest contiguous prairies on the North American continent. Overall we observed evidence of seven amphibian species breeding in the Sheyenne National Grasslands. Species with ubiquitous distributions (Boreal Chorus Frogs) or species that occurred infrequently (Canadian Toads and Great Plains Toads) had little variance in occupancy. However, Northern Leopard Frogs, Wood Frogs, and Gray Treefrogs exhibited occupancy relationships with wetland and landscape variables. Our results establish a baseline understanding of current prairie amphibian–habitat relationships. Furthermore, they indicate that integrating local and landscape variables into occupancy models that account for spatial autocorrelation can provide a better understanding of amphibian ecology, and can inform conservation and restoration programs.

Key words: Frog, Hyla; Landscape ecology, Lithobates; Sheyenne National Grasslands; Toad

Worldwide, both terrestrial and aquatic ecosystems have been dramatically altered from their natural states. Draining and filling of wetlands for agricultural crop production are the primary causes of wetland losses, with estimated total losses near 30% in the United States alone (Dahl 1990). The majority of native terrestrial habitats surrounding wetlands have also been modified. For example, within North America, 82–99% of the original tall- and mixed-grass prairie has been converted to agricultural use (Samson and Knopf 1994). Most of the original grassland no longer exists, and much of what remains is no longer contiguous. In the face of such habitat reduction and fragmentation, understanding which remaining portions support aquatic and terrestrial wildlife populations is essential for their conservation.

Amphibians require both aquatic and terrestrial habitats, which increases their susceptibility to population fluctuations in disturbed environments. Worldwide reports of amphibian decline and threats of extinction (Houllahan et al. 2000; Stuart et al. 2004; Adams et al. 2013) have increased the urgency for understanding how amphibians interact with their local and regional environment (Cushman and McGarigal 2002; Trenham and Shaffer 2005; Cushman 2006). Declines have been associated with several biotic and abiotic factors (Stuart et al. 2004; Beebee and Griffiths 2005); however, the most consistent factors appear to be habitat loss and fragmentation (Cushman 2006).

Although breeding wetlands are obvious requirements for amphibian populations (Semlitsch 2000; Petranka et al. 2007), several authors note the importance of the surrounding upland habitat for maintaining ecological connectivity among breeding sites (Semlitsch 2000; Cushman 2006; Denoël and Lehmann 2006). Converting upland habitat into croplands often reduces amphibian dispersal (Joly et al. 2001), which can lead to reduced genetic diversity and increased local extinctions (Sjögren 1991; Semlitsch and Bodie 2003; Johansson et al. 2005; Hamer and Mahoney 2010). Through a combination of short-term (e.g., Houllahan and Findlay 2003; Van Buskirk 2005; Denoël and Lehmann 2006) and long-term studies (e.g., Hecnar and M’Closkey 1996; Skelly et al. 1999; Gibbs et al. 2005; Petranka et al. 2007), it has become apparent that landscapes surrounding wetlands should be conserved to support amphibian populations.

Given the depletion of native prairie and prairie wetlands in the United States, we have a limited basis for understanding how amphibian populations functioned across prairie landscapes prior to modification. In North Dakota alone, 71.9% of native mixed grass, 99.9% of native tall grass, and 49% of the prairie wetlands have been lost to human development (Samson and Knopf 1994). Further, remaining prairie generally consists of small isolated fragments (Mushet et al. 2014). The Sheyenne National Grasslands (SNG) of North Dakota provides a unique opportunity to study amphibian distributions in a relatively contiguous prairie landscape. The SNG is approximately 275 km² of private and public grassland located on the western boundary of the tallgrass prairie ecoregion in southeastern North Dakota, making it one of the largest remaining portions of contiguous prairie in North America (Cunningham and Johnson 2006).

Based on the life history of wetland-breeding amphibians, we hypothesized that amphibian distributions depend on a range of environmental factors spanning multiple ecological scales. The first level is the aquatic environment where amphibians breed. Wetlands typically vary in water chemistry, temperature, hydroperiod, vegetation, and predators, all of which are known to influence amphibian reproductive success (Semlitsch 2000). Low pH can result in reduced tadpole survival (Sparling et al. 1995), whereas high specific conductance (SC) has been positively correlated to amphibian occupancy (Klaver et al. 2013). Hydroperiod is critical for tadpole development and can affect the predator community, including fish species that reduce egg or tadpole survival (Skelly 1996; Hecnar and M’Closkey 1997; Relyea 2001; Amburgey et al. 2014). Additionally, wetland vegetation

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provides oviposition sites for female frogs, as well as cover and detritus for tadpoles (Egan and Paton 2004; Schmutzer et al. 2008).

After metamorphosis, most amphibian species become physiologically adapted to terrestrial environments. Several studies have demonstrated reduced dispersal in agricultural lands (Pope et al. 2000; Joly et al. 2001; Cosentino et al. 2011). Roads and rivers can also serve as barriers to dispersal, or are sources of direct mortality (Gibbs 1998; Carr and Fahrig 2001; Hels and Buchwald 2001; Cosentino et al. 2014). In contrast, intact forests and prairies likely provide habitat for overwintering and dispersal (Guerry and Hunter 2002; Denoël and Lehmann 2006). Thus, we expect several landscape features to affect amphibian occupancy (Semlitsch 2000).

To address how local and landscape characteristics affect amphibian breeding site use, we used a combination of field observations, geographic information systems, and hierarchical statistical modeling to identify the factors that predict amphibian detectability and occupancy within the SNG. Occupancy is defined as the probability of a site (sampling unit) being occupied by the species (MacKenzie et al. 2009). Detectability addresses the challenge of estimating occupancy in the face of imperfect detection (MacKenzie et al. 2009). In our study, an occupied site refers to a wetland where breeding activity has occurred. Identifying wetland characteristics and landscape features that contribute to amphibian occupancy can provide a better understanding of the requirements of various amphibian species in the Great Plains, and can help inform future prairie conservation and restoration efforts.

MATERIALS AND METHODS
Study Area

The north unit of the SNG is ~275 km² of private and public grassland located on the western boundary of the tallgrass prairie ecoregion in southeastern North Dakota; it once functioned as a river delta for glacial Lake Agassiz. The Sheyenne River flows eastward near the northern border of the SNG and is responsible for the landscape structure of this region. Bordering the Sheyenne River is a contiguous stand of riparian deciduous forest that makes up about 5% of the overall landscape (Manske and Barker 1988). Wetlands in this area tend to be mostly shaded, permanent, oxbow lakes. South of the river, the landscape transitions from forest to rolling sandhills that make up nearly 15% of the overall landscape (Manske and Barker 1988). The rolling sandhills transition into hummocky sandhills, which gradually become deltaic plains. The hummocky sandhills make up the largest proportion of the landscape (~50%) and contain the highest density of ephemeral wetlands (Manske and Barker 1988). The deltaic plains make up the remaining portion of the SNG (~30%; Manske and Barker 1988).

The primary vegetation in the SNG is a combination of tall and mixed-grass prairie species, mainly Kentucky bluegrass (Poa pratensis), western wheatgrass (Pascopyrum smithii), blue grama ( Bouteloua gracilis), and big bluestem (Andropogon gerardii; Lym et al. 1997). Although this region contains a high diversity of vegetation types, it is not pristine. Many areas are severely impacted by cattle grazing, which is the main land use on the SNG. Additionally, the landscape is interspersed with tree rows and remnant farm field drainages. Leafy spurge (Euphorbia esula) has invaded and outcompeted many native plants and is now at such a high density that it has reduced forage production and grazing capacity in the region (Lym et al. 1997). Agricultural land completely surrounds the SNG, making the grassland an island of prairie habitat.

Previous amphibian surveys (2001–2002) identified six amphibian species inhabiting the SNG (Bly 2004): Northern Leopard Frogs ( Lithobates pipiens ), Wood Frogs ( Lithobates sylvaticus ), Gray Treefrogs ( Hyla versicolor × chrysoscelis ), Boreal Chorus Frogs ( Pseudacris maculata ), Canadian Toads ( Lithobates catesbeianus ), and Barred Tiger Salamanders ( Ambystoma maculatum ).

Sampling Design

To determine the status of amphibian breeding populations, we conducted an occupancy study with repeated sampling. Amphibians require wetlands to survive and reproduce, making wetlands appropriate, and hypothetically independent, sampling units. Because there is a logistical trade-off between spatial coverage and repeated sampling (MacKenzie and Royle 2005; Bailey et al. 2007), we employed a two-stage sampling design with systematically selected regions broadly distributed across the landscape and local sampling within those regions (Fig. 1). We surveyed known wetlands within previously established regions (Bly 2004); we also surveyed previously unsampled wetlands in additional regions based on geographic coverage, distance from other regions, and ease of access. The previously unsurveyed wetlands were found by searching on the ground with the aid of aerial maps. Broad-scale systematic sampling maximized geographic coverage and permitted assessment of spatial distribution patterns across landscape-level gradients.
Sampling locally within primary sampling regions allowed us to estimate occupancy patterns at the wetland scale where demographic processes (i.e., immigration, emigration, extinction, colonization) are likely to occur.

Field Methods

We sampled 141 wetlands in the summer of 2009 and 126 wetlands (a random subset of the 141 wetlands surveyed in 2009) in the summer of 2010 (Fig. 1). Wetlands were surveyed 1–5 times in 2009 and 1–3 times in 2010. At each site, we assessed the presence of calling males, egg masses, tadpoles, and metamorphs of all anuran species encountered using visual encounter surveys (Doan 2003) and dip-net sampling for larval stages. We recorded the geographic location (UTM coordinates) with a Garmin GPSmap76Cx. Because the entire perimeter of each site was surveyed, the number of dip-net samples and time spent at each site was proportional to the perimeter of each wetland. Habitat variables included the presence of emergent vegetation and aquatic vegetation, assessed visually. Additionally, the presence of aquatic insects was recorded during dip-net sampling. We measured water specific conductance (μs/dL) and pH with the use of a handheld water chemistry device (YSI 63/10 FT). We also recorded if the wetland was an artificial cattle pond and if the wetland completely dried during either summer prior to the period of metamorphosis (i.e., end of August). Additional covariates tested in detectability analyses included date (i.e., Julian date), time of day, air temperature (°C, measured in the shade at hip height), and average wind speed (kph, over 30 s). Water measurements were taken after the survey to minimize disturbance and maximize amphibian detectability.

Geographic Information Systems (GIS) Procedures

Using ESRI ArcMap v9.3, we determined the wetland basin area and perimeter for each sampled wetland from 1-m-resolution infrared aerial imagery acquired from the National Agriculture Imagery Program (https://gdg.sc.egov.usda.gov/). The landscape was classified from multiple GIS layers, including manually digitized tree cover, wetlands from the U.S. National Wetlands Inventory (http://www.fws.gov/wetlands/), cultivated crops from U.S. Geographic Survey landcover data (https://gdg.sc.egov.usda.gov/), and road data acquired from the state of North Dakota’s geographic information system (GIS) hub (https://www.nd.gov/gis/). We superimposed GIS buffers around each wetland to quantify terrestrial characteristics. Because amphibian species have different habitat requirements and dispersal rates, different species are likely to be influenced at different spatial scales (Bosch et al. 2004; Smith and Green 2005). Thus, we built a series of nested buffers (at radii of 2000 m, 1000 m, 500 m, 250 m, 100 m, and 50 m from the wetland edge) around each site for potential use in species-specific occupancy models. We then calculated the proportion of each land-cover type (i.e., trees, water, number of wetlands, cultivated crops) within each buffer, and the distance to the nearest road, cultivated field, and tree cover. In addition to landscape features, demographic connectivity must be accounted for when one is attempting to understand spatial population structure (Pope et al. 2000; Marsh and Trenham 2001; Schmidt and Pellet 2005). To quantify a potential measure of demographic connectivity for each species, we calculated the distance to the nearest wetland observed as being occupied by that species.

Because they are proportions, we expected each landscape variable to be structurally correlated to all other landscape variables. However, there were no correlations between landscape variables. Instead, all intrasite landscape buffers (i.e., from 50 to 2000 m) were highly correlated ($r^2 > 0.5$). Because of our two-stage sampling design, buffers of 250 m or larger contained appreciable areas of overlap with neighboring wetlands and were nonindependent. Thus, we limited our analysis to the proportion of each landscape type within 100-m buffers.

Spatial Autocorrelation

One major issue with spatial analyses is that measurements taken at different locations are often not spatially independent (Legendre 1993). Biological entities that are closer together in space and time tend to be more similar than those that are further apart (Tobler 1970; Miller 2004). Analyses that do not account for spatial autocorrelation in response variables tend to have inflated Type I errors, which might lead to spurious or misleading results (Kühn 2007). Because our wetlands varied in proximity to each other (min–max = 10–7000 m), we used spatial eigenvector mapping to account for spatial autocorrelation (Borcard and Legendre 2002; Dormann et al. 2007). Spatial eigenvector mapping accounts for spatial patterns over a wide range of scales and establishes a set of explanatory variables that can be used in regression analyses (i.e., spatial filters; Borcard and Legendre 2002; Borcard et al. 2004; González-Mégas et al. 2005). Spatial filters are based on distance matrices, are arranged from broad to fine scale, and are used to capture purely spatial information (Borcard and Legendre 2002). We used program SAM (Spatial Analysis in Macroeology, v4.0; Rangel et al. 2010) to calculate a distance matrix and spatial eigenvectors. We tested for spatial autocorrelation in predictor and response variables with the use of Moran’s I estimated in SAM. Statistical significance testing was performed with the use of permutation tests (1000 permutations). To account for spatial patterning, in general, and reduce effects of spatial autocorrelation in response variables in regression analyses, we used spatial filters that were significantly correlated with species-specific, detectability-corrected occupancy probabilities. Correlations were calculated prior to constructing occupancy models. Any spatial filters that correlated to observed occupancy were allowed to compete with local and landscape variables. Significant eigenvectors are indicators of the spatial scales where spatial autocorrelation is present (Dimiz-Filho and Bini 2005). Thus, local and landscape variables that remained in occupancy models after the spatial filters are added were considered spatially independent predictors of species occupancy.

Statistical Methods

We modeled the detection and occupancy probabilities of each species in relation to local wetland characteristics, landscape variables, and spatial filters with the use of program PRESENCE v8.9 (Hines 2006). Because of reduced sampling in 2010, multiseason occupancy models would not converge. Similarly, given the relatively brief windows in which most life stages could be surveyed, and
that individuals representing each species breed in more than a single year, considering data isolated by year would produce an artificially inflated sample size. Consequently, detectability should be interpreted to mean probability of detection of at least one breeding stage over 2 yr, although true detection probability varies among stages and possibly among years. Our response variable of primary interest was occupancy by breeding animals, conditioned on detectability, represented by any stage associated with breeding activity over the 2-yr period. We acknowledge that our model might be biased with respect to its handling of data that represent Boreal Chorus Frogs.

Although landscape variables did not change between years, local conditions did. Accordingly, we incorporated survey-specific detectability covariates, including Julian date, time of day, air temperature, and wind speed. Although pH and SC varied across wetlands, individual sites exhibited relatively consistent values. Thus, SC (mean ± 1 SD = 505.3 ± 249.9 μS/dL; range: 5.5–1270 μS/dL) and pH (mean = 8.40 ± 0.33; range: 7.51–9.48) were each averaged among repeated samples at each individual site. To understand better the relative effect of each variable to one another, we chose the option in program PRESENCE to standardize all variables to a mean of 0 ± 10 SD.

Estimation of occupancy models for each species required several steps. First, we estimated correlations among predictor variables to check for potential collinearity. Local wetland variables were correlated, including aquatic vegetation, aquatic insects, emergent vegetation, pH, and SC. Rather than arbitrarily eliminating correlated variables from analyses, we used a principal-components analysis on local wetland characteristics. The first three axes of the wetland characteristics (local wetland factor [LWF] 1, 2, and 3) accounted for 75.8% (34.9, 22.5, and 18.4, respectively) of the variance and were used as predictor variables. Interpretation of these composite variables was based on inspection of factor loadings on the principal component axes. Nearly 92% of the variable contribution to LWF1 consisted of the presence or absence of aquatic vegetation (25.4%), aquatic insects (37.5%), and emergent vegetation (28.9%); negative values corresponded to wetlands with aquatic vegetation, aquatic insects, and emergent vegetation, whereas positive values indicated their absence. Just over 97% of the variable contribution to LWF2 consisted of pH (33.6%) and SC (63.5%), where negative values indicated low pH and high SC and positive values indicated high pH and low SC. LWF3 consisted of a combination of aquatic vegetation (31.6%), pH (47.2%), and SC (18.9%), where positive values indicated the presence of aquatic vegetation, high pH, and high conductivity.

Rather than restricting our inference to a single best occupancy model, we used information-theoretic methods, and ranked models based on sample-size-corrected Akaike Information Criterion (AICc) scores (Burnham and Anderson 2002). We used best subsets regression models incorporating hypothesized candidate variables for variable selection (Hosmer et al. 2013). Unlike model-selection approaches that use backward, forward, or stepwise selection, best-subsets models yield consistent results regardless of the order in which models are computed and do not rely on P values to determine whether an explanatory variable should be included or excluded (Burnham and Anderson 2002).

**Fig. 2**—Seasonal breeding behavior of (A) Northern Leopard Frogs, (B) Wood Frogs, (C) Gray Treefrogs, (D) Boreal Chorus Frogs, and (E) Canadian Toads in the Sheyenne National Grasslands. Indicators of breeding activity included the presence–absence of calling males (Calls), egg masses (Eggs), tadpoles (Tads), metamorphic frogs (Meta), and young-of-the-year juveniles (Juvs). Observations of breeding activity are represented as circles (2009) or squares (2010) that are offset for visualization purposes only. Horizontal lines represent the earliest and latest observations of each breeding activity and are presented for visualization purposes only. Vertical hashmarks on the x-axes indicate actual wetland survey dates.

hence few or no repeated visits within calling and egg-laying periods, multistate occupancy models (where states included calling males, eggs, tadpoles, and metamorphs) would not converge. Thus, we used single-season occupancy models of combined data from 2009 and 2010, and treated the presence or absence of any evidence of breeding activity (i.e., calling, eggs, tadpoles, metamorphs) at each wetland (n = 141) as a single binary response (Fig. 2). This method inherently violates the assumption of a closed population and is not consistent with the population biology of the focal species, which likely have interannual variation. With the exception of Boreal Chorus Frogs, however, occupancy patterns did not change much between years, and pooling data over a 2-yr period had negligible effect on interpretations stemming from our analyses. Additionally, considering...
Table 1.—Amphibian species detected within the Sheyenne National Grasslands. Top detectability models, naïve occupancy, detectability-conditional occupancy, and standard errors are presented for each species. Great Plains Toads were not detected breeding at any of our specific sites and we did not seine for Barred Tiger Salamanders; thus, occupancy estimates are not presented for these two species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Top detectability model</th>
<th>Naïve $\psi$</th>
<th>Conditional $\psi$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Leopard Frogs</td>
<td>$p(Survey+Date+AirTemp+WindSpeed)$</td>
<td>60.28</td>
<td>76.90</td>
<td>5.16</td>
</tr>
<tr>
<td>Wood Frogs</td>
<td>$p(Survey+Date+Time+AirTemp)$</td>
<td>22.70</td>
<td>34.59</td>
<td>6.67</td>
</tr>
<tr>
<td>Gray Treefrogs</td>
<td>$p(Survey+Date+Time+AirTemp)$</td>
<td>23.87</td>
<td>34.32</td>
<td>4.82</td>
</tr>
<tr>
<td>Boreal Chorus Frogs</td>
<td>$p(Survey)^a$</td>
<td>73.76</td>
<td>89.71</td>
<td>5.11</td>
</tr>
<tr>
<td>Canadian Toads</td>
<td>$p(1)^a$</td>
<td>14.18</td>
<td>29.95</td>
<td>9.26</td>
</tr>
</tbody>
</table>

* More complex models would not converge.

In general, models that have a $\Delta$AICc score within 2.0 of the top model are considered to have similar levels of support (Burnham and Anderson 2002). The majority of the top-ranked models were very similar in model structure. Thus, parameter estimates were calculated with the use of model averaging based on AICc weights for all models that had a $\Delta$AICc within 2.0 of the top model (Mazerolle 2006).

After computing top models, we computed three null models to identify the relative importance of including detectability covariates and/or occupancy predictor variables. The three null models included: (1) the top model with no survey-specific detectability covariates; (2) no occupancy predictor variables, but the inclusion of survey-specific detectability covariates; and (3) no detectability covariates and no occupancy predictor variables. Finally, we assessed model fit by inspection of model residual deviances (deviance after accounting for covariates) relative to the deviance associated with the null model with no covariates (Hilbe 2009). This assessment provided some insight into the magnitude of variability in occupancy actually accounted for by the final model.

**RESULTS**

**Occupancy Estimates**

Overall, we detected seven amphibian species in the SNG (Table 1). In addition to the six amphibian species encountered during previous surveys, we also found the Great Plains Toad (*Anaxyrus cognatus*). Although we were unable to detect breeding activity of Great Plains Toads in any of the wetlands surveyed, juveniles and adult Great Plains Toads were observed across the landscape and breeding choruses were heard several nights, indicating that Great Plains Toads are breeding somewhere in the SNG.

In all cases, naïve occupancy estimates were lower than occupancy estimates that accounted for imperfect detection (Table 1). For species with intermediate occupancy estimates (i.e., Northern Leopard Frogs, Wood Frogs, Gray Treefrogs; Table 1), models containing predictor variables and accounting for survey-specific detection probability covariates greatly improved model support relative to null models (Table 2). Because of the nearly ubiquitous occurrence of Boreal Chorus Frogs over the 2 yr, neither local or landscape variables could predict Boreal Chorus Frog occupancy and models would not converge. Models would also not converge for Canadian Toads because of the low numbers of breeding observations (Table 1; Fig. 2). Although local, landscape, and population variables improved model support based on AICc scores, model deviances indicate that there remains considerable unexplained variation in occupancy. Based on the ratio of the reduction in deviance relative to null deviance, the top model for Northern Leopard Frogs accounted for 28.15% of the variation in occupancy, whereas the top models for Gray Treefrogs and Wood Frogs accounted for 12.56 and 7.24%, respectively.

**Local and Landscape Analyses**

Spatial patterning (i.e., variation explained solely by location) was present in occupancy models and was primarily evident over broader range spatial filters (i.e., 1–10). However, fine-scale spatial patterning (spatial filter 12) was present in Wood Frog occupancy models.

Local wetland variables were consistently represented in occupancy models. All species exhibited negative relationships of occupancy with LWF1 (i.e., PC 1), indicating that all species preferred wetlands with aquatic vegetation, emergent vegetation, and aquatic insects (Table 3). LWF3 was present in all top models for Northern Leopard Frogs and Wood Frogs. This factor had a positive beta estimate, indicating increased occupancy with higher pH and SC, and with the presence of aquatic vegetation (Table 3; Fig. 3). LWF2 was represented in the highest-ranked models for Gray Treefrogs, and the positive beta estimate indicated a positive correlation with higher pH and lower SC. Occupancy of Northern Leopard Frogs and Wood Frogs was lowest in wetlands that dried during our study. Additionally, occupancy of Gray Treefrogs decreased in the artificial cattle ponds.

Landscape variables were consistently included in top models. Occupancy was positively related to forested habitats. The occupancy of Northern Leopard Frogs and Wood Frogs increased with the proportion of tree cover (Table 3; Fig. 4A,C). Although model selection did not indicate that the proportion of tree cover was an important predictor of occupancy by Gray Treefrogs, the likelihood of occupancy by this species decreased with increasing distance from tree cover (Table 3; Fig. 4E). Occupancy of Wood Frogs and Gray Treefrogs increased with the surrounding number of wetlands (Table 3; Fig. 4D). Occupancy of Northern Leopard Frogs decreased with increasing distance from the Sheyenne River (Table 3; Fig. 4B), whereas that for Wood Frogs was positively correlated with distance from the river. Occupancy of Northern Leopard Frogs was also negatively associated with distance from roads. Gray Treefrogs were the only species to increase when nearby wetlands were occupied by conspecifics (Table 3; Fig. 4F).

**DISCUSSION**

We observed amphibian occupancy relationships with a variety of factors, including breeding habitat and landscape...
factors. In comparison, we suggest that research focusing solely on local conditions reduces the accuracy for predicting or describing amphibian distributions (Mazerolle and Villard 1999), and might overestimate the potential of a landscape to maintain populations. Likewise, focusing only on landscape variables might not account for the specific properties of quality breeding habitats. Our results indicate that integrating local and landscape factors into occupancy models that account for spatial autocorrelation provides a better understanding of amphibian ecology and conservation. Although these models are correlative in nature, they provide statistical support for the importance of factors that influence amphibian populations in the SNG.

Within the SNG, wetland-breeding amphibians consistently selected wetlands containing aquatic and emergent vegetation. This is not surprising because aquatic vegetation provides cover, food, and oviposition sites for amphibians (Schmutzer et al. 2008). Cattle ponds, excavated basins that provide water for cattle, are common in the SNG and typically lack vegetation. Although cattle ponds are poor breeding habitats for anurans, they are commonly used by Barred Tiger Salamanders in this region (Bly 2004). Because these wetlands are designed to be permanent, they also provide potential habitat for fish species that can negatively influence amphibian populations (Heecnar and M’Closkey 1997). We did not detect any fish in the cattle ponds we surveyed, and the majority of wetlands on the grassland are ephemeral. Thus, fish do not appear to be a major concern for amphibians in the SNG. It is also not surprising that amphibians occupied wetlands with high pH and high SC because acidic waters have been shown to be detrimental to larval amphibian survival (Gosner and Black 1957; Freda 1986; Sparling et al. 1995), and high SC has been previously correlated to amphibian occupancy (Klaver et al. 2013).

The SNG has a large number of wetlands, many of which are suitable for amphibian breeding activity. Many of those wetlands remain unoccupied, however, likely because of the composition of the surrounding landscape and the breeding behavior of amphibians. Although occupancy by Boreal Chorus Frogs and Canadian Toads could not be modeled because of their ubiquitous or restricted distributions, respectively, most species were nonrandomly distributed. Some displayed broad-scale trends in occupancy, and others showed fine-scale variation. The Sheyenne River was one of the most important landscape features influencing species distributions at broad scales. Occupancy of Northern Leopard Frogs was strongly associated with the river, whereas that of Wood Frogs showed the opposite trend. Although amphibians might not be directly influenced by the river, there are many landscape changes that correspond to

Table 2.—Model selection results for amphibian occupancy. Top models (ΔAICc < 2) and null models (treating occupancy and/or detectability as constant) are presented. Models for Boreal Chorus Frogs and Canadian Toads could not be computed. ΔAICc = sample size-corrected Akaike Information Criterion; w = model weight; −2L = −2(log likelihood); LWF = local wetland factor; Dist = distance to; SF = spatial filter.

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>ΔAICc</th>
<th>w</th>
<th>K</th>
<th>−2L</th>
<th>Model deviance</th>
</tr>
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<tbody>
<tr>
<td>Northern Leopard Frogs</td>
<td>Ψ(LWF1 + LWF3 + Dried + Trees100m + DistRiver + SF5 + SF7), p(Survey + Date + AirTemp + WindSpeed)</td>
<td>0</td>
<td>0.73</td>
<td>21</td>
<td>564.99</td>
<td>575.51</td>
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<td></td>
<td>Ψ(LWF1 + LWF3 + Dried + Trees100m + DistRoad + DistRiver + SF5 + SF7), p(Survey + Date + AirTemp + WindSpeed)</td>
<td>2</td>
<td>0.27</td>
<td>22</td>
<td>564.17</td>
<td>552.16</td>
</tr>
<tr>
<td></td>
<td>Null 1: Ψ(1), p(Survey + Date + AirTemp + WindSpeed)</td>
<td>36.19</td>
<td>0.00</td>
<td>14</td>
<td>619.61</td>
<td>701.27</td>
</tr>
<tr>
<td></td>
<td>Null 2: Ψ(LWF1 + LWF3 + Dried + Trees100m + DistRiver + SF5 + SF7), p(1)</td>
<td>111.18</td>
<td>0.00</td>
<td>9</td>
<td>706.56</td>
<td>650.39</td>
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<tr>
<td></td>
<td>Null 3: Ψ(1), p(1)</td>
<td>158.75</td>
<td>0.00</td>
<td>2</td>
<td>769.41</td>
<td>801.02</td>
</tr>
<tr>
<td>Wood Frogs</td>
<td>Ψ(LWF3 + Trees100m + Wetlands100m + SF3), p(Survey + Date + Time + AirTemp)</td>
<td>0</td>
<td>0.31</td>
<td>18</td>
<td>237.7</td>
<td>41.39</td>
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<td></td>
<td>Ψ(LWF3 + Trees100m + Wetlands100m + DistRiver + SF3), p(Survey + Date + AirTemp)</td>
<td>0.66</td>
<td>0.22</td>
<td>19</td>
<td>235.69</td>
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<td></td>
<td>Ψ(LWF3 + Trees100m + Wetlands100m + SF3), p(Survey + Date + AirTemp)</td>
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* Indicates null deviance. 
increasing distance from the river. For example, forest and cropland density were highest near the river, whereas wetland density increased with distance from the river.

Northern Leopard Frogs, Wood Frogs, and Gray Treefrogs were all positively correlated with tree cover. Tree cover provides important habitat for hibernation, foraging, and dispersal (Guerry and Hunter 2002; Denoël and Lehmann 2006). Interestingly, the proportion of surrounding tree cover was important in models for Northern Leopard Frogs and Wood Frogs, whereas the proximity to tree cover was important in occupancy models for Gray Treefrogs. This is likely a result of differences in dispersal ability, where ranids are more capable than hylids of long-distance dispersal (Smith and Green 2005).

Within the portion of the SNG sampled, croplands were generally concentrated near the river, and were either near forested areas or associated with tree rows. However, none of the species showed positive or negative relationships with agricultural land. It is important to note that we did not sample within agricultural lands, so our inference is limited to wetlands near croplands. Previous reports have observed increased parasite infections in Northern Leopard Frogs occupying agricultural sites (Christin et al. 2003; King et al. 2008; Rohr et al. 2008a,b). Thus, it is important to note that previous surveys of the parasitic worms in Northern Leopard Frogs and Wood Frogs from the SNG did not find any evidence of unusually high infection intensities (Goldberg et al. 2001; Bly 2004; Gustafson et al. 2013).

A potentially counterintuitive relationship presented by our results is the positive association of Northern Leopard Frogs with roads. There is only one paved road in the SNG that conveys steady traffic flow; the majority of roads are gravel and vehicle traffic is light. In fact, in both summers of our study period, several roads in the grassland were flooded and could not be traveled. Thus, proximity to roads generally refers to proximity to gravel roads (often minimum maintenance roads), which are much less likely to impact amphibian populations through direct mortality as has been observed by other researchers (Carr and Fahrig 2001; Hels and Buchwald 2001; Cosentino et al. 2014). Instead, inundated road ditches may be serving as corridors for dispersal. In both years, the majority of ditches contained standing water and could function to facilitate dispersal along roadsides.

In addition to wetland and landscape characteristics, we also expected demographic connectivity would be an important determinant of amphibian occupancy. We used distance to the nearest occupied wetland (for each species) as a measure of connectivity. Gray Treefrogs were the only species to exhibit relationships with nearby occupied wetlands, and for them, occupancy was higher when neighboring wetlands were also occupied. This finding indicates that breeding sites for Gray Treefrogs are more clustered than those of other species. Additionally, their affinity for tree cover and relatively low dispersal ability likely contributed to this pattern. For species with stronger dispersal capabilities, the SNG may simply contain a high

**TABLE 3.—Variables represented in top occupancy models (ΔAICc < 2).** Relative variable importance, model-averaged beta parameter estimates, beta standard errors and beta 95% confidence intervals are presented. For direct variable comparisons, all variables were standardized to 0 with equal intervariable variance (±10 SD). Higher absolute values of beta estimates indicate a greater effect on occupancy. LWF = local wetland factor; Dist = distance to; SF = spatial filter.

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>RVI</th>
<th>β</th>
<th>SE</th>
<th>95% confidence interval</th>
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enough density of wetlands that connectivity across the landscape was not limiting to occupancy, at least during the wet conditions that prevailed during the time period of this study.

Spatial filters were commonly included in occupancy models for all species and helped to reduce bias caused by spatial autocorrelation. However, interpreting geographic variables in regression models is not straightforward (Hawkins and Diniz-Filho 2004). The inclusion of broad-scale filters in top models might result from spatial autocorrelation attributable to broad-scale patterns, such as soil type, hydrology, or other landscape factors. In contrast, the meso- and fine-scale filters reflect environmental patterns and/or demographic processes at more local scales (Diniz-Filho and Bini 2005). In many cases, spatial filters accounted for a substantial proportion of the overall variance, supporting the conclusion that spatial processes, either related to unmeasured environmental factors or patterns of demographic connectivity, were occurring at those scales (Borcard et al. 2004).

It is important to note that, although we recorded strong support for occupancy relationships with several measured variables, much of the variance in anuran occupancy could not be attributed to particular variables. This outcome indicates that our models lack important predictors that we did not measure, or that amphibian population processes are somewhat stochastic across the landscape. Both reasons likely contribute to unexplained variation in occupancy.

Fig. 4.—Probability of amphibian (A, B = leopard frog; C, D = Wood Frog; E, F = Gray Treefrog) occupancy in relation to landscape factors. Site-specific estimates of occupancy (dots), predicted lines (solid line), and 95% confidence intervals (dashed lines) are presented. Factors with absolute value beta estimates greater than 0.4 are presented.
Increasing the number of wetlands surveyed, the number of repeated sampling events per wetland, and the spatial extent or temporal time frame of surveys could help improve model fit. Additionally, combining data from both study years could have masked some of the intrawetland variation.

**Conservation Implications**

The current state of amphibian declines makes it essential to understand how breeding populations of amphibians are structured across landscapes (Pechmann et al. 1991; Houblon et al. 2000; Kiesecker and Skelly 2001; Stuart et al. 2004). During the course of our study, we did not detect any amphibian mass mortalities and have no evidence to suggest these populations are unusually low in abundance. In general, the SNG supports a high density of amphibians and provides sufficient breeding habitat. Distributions did not depend solely on local or landscape factors. Instead, models that integrated several factors generally had better support. Thus, amphibian conservation programs should extend beyond individual breeding wetlands to include the surrounding landscape (reviewed in Semlitsch 2000). Persistence of amphibian populations depends both on availability of high-quality aquatic habitat across a landscape and terrestrial habitat suitable for juvenile and adult dispersal and survival.

Many of the wetlands in the SNG were not used for breeding, but might still be important for supporting amphibian populations over long time frames. Some species are capable of long-distance movements (Smith and Green 2004) and have the potential to colonize new areas. In addition, wetlands that are not used for reproduction might have other ecological roles in sustaining amphibian populations (e.g., overwintering and foraging) and could become important breeding sites under different environmental circumstances. Thus, unoccupied wetlands and the habitat connecting wetland communities should be maintained. Our results establish a baseline understanding of prairie amphibian–habitat relationships at the wetland and landscape scale. Our data can help future researchers develop hypotheses particular to a given species pertaining to its distribution. Additionally, our data can help conservation and restoration ecologists establish specific wetland and landscape goals to better support amphibians in the SNG and potentially across the Great Plains.

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**Literature Cited**


