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Relationships Between Isolating Land Use and Amphibian Populations in Sub-Boreal Peatlands of the Midwestern United States

Jeana Rose Albers

Minnesota State University - Mankato

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Relationships between isolating land use and amphibian populations in sub-boreal
peatlands of the Midwestern United States

By

Jeana Albers

A Thesis Submitted in Partial Fulfillment of the

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Relationships between isolating land use and amphibian populations in sub-boreal
peatlands of the Midwestern United States

Jeana Albers

This thesis has been examined and approved by the following members of the student's
committee.

November 10th, 2014

Dr. John D. Krenz, Advisor

Dr. Bradley J. Cook

Dr. Christopher T. Ruhland

ABSTRACT

Relationships between isolating land use and amphibian populations in sub-boreal peatlands of the Midwestern United States

Jeana Albers

Master of Science in Biology

Minnesota State University, Mankato

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Successful conservation efforts of amphibians depend on the knowledge of habitat preferences because the biggest threat to amphibian populations is considered to be habitat loss. Sub-boreal peatlands in the Midwest may be a refuge for amphibian populations, but little is known about the flora and fauna of these peatlands. My study examined amphibian species richness and species diversity in 17 sub-boreal peatlands in Minnesota and Wisconsin in 2011 and 2012. I assessed the relationship between land use of lands adjacent to peatlands and use of those peatlands by amphibian populations at three spatial scales by examining landscapes surrounding peatlands in circles with radii of 500, 1000, and 2500 m. I tested for linear relationships of both species richness and species diversity with habitat variables including peatland area, aqueous pH, geographic isolation, canopy cover, and the proportions of forest and isolating land use (agricultural and urban) in the three radii. I hypothesized that high levels of isolating land use isolate peatlands via habitat loss and degradation of surrounding land. I predicted that species richness and diversity would be low in peatlands that experienced high levels of agricultural and urban land use because they were more isolated. I found no support for my prediction for the correlation of isolating land use with lower amphibian diversity and

richness, but isolation was an important indicator of species richness, with more isolated peatlands having fewer species present. Canopy cover was also an important predictor of species richness at all three spatial scales in 2012, with greater canopy cover correlating with lower species richness. Other studies corroborate my findings in regard to isolation and canopy cover having negative effects on amphibian populations, and because of this and my findings, I suggest that conservation efforts for amphibians in sub-boreal peatlands be focused on maintaining connectivity between peatlands, and prioritize those that have little canopy cover to be refuges for remaining populations.

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INTRODUCTION

The decline in global amphibian populations has gained worldwide attention (Wyman 1990, Blaustein 1994, Genet and Sargent 2003, Mazerolle 2003). It is likely that there is no single cause, but the biggest threats are thought to be habitat loss and habitat degradation (Knutson et al. 1999, Wilson and Dorcas 2003). The threat of habitat loss for amphibian populations has caused amphibian conservation efforts to be focused on wetland protection, due to their high productivity and storage of water, necessary for completion of amphibian life cycles (Oldfield and Moriarty 1994, Skelly et al. 1999, Paillisson et al. 2002, Wilson and Dorcas 2003, Muenz et al. 2006). In addition to wetland conservation, another component that is important in supporting amphibian populations is the surrounding buffers that can be classified as forest, with high forest coverage correlated with greater amphibian richness and diversity (Skelly et al. 1999, Houlahan and Findlay 2003, Eigenbrod et al. 2008) due to the provision of moist organic litter that protects amphibians from desiccation when foraging, dispersal, and migration (deMaynadier and Hunter 1999). However, canopy cover over a wetland does not facilitate successful reproduction because canopied areas have lower water temperatures and less solar irradiance than what is necessary for larval development (Skelly et al. 2002, Halverson et al. 2003, Schiesari 2006). Algae that amphibian larvae use as a food source are less abundant in shaded peatlands (Skelly et al. 2002). Even if the wetland does not have high surrounding forest cover, a terrestrial “buffer,” the land that is located between a wetland and the upland used for human uses (*i.e.*, residential, agriculture), is important for maintaining amphibian populations, and appropriate buffer widths have

attracted the attention of regulatory agencies (Semlitsch 1998, Semlitsch and Bodie 2003).

Regulatory agencies have found that a buffer width of 30 to 60 meters surrounding a wetland is effective in protecting water quality (Castelle et al. 1994, Semlitsch and Bodie 2003), but studies suggest that at least 500 m of terrestrial buffer is required for amphibians (Findlay and Houlihan 1997, Semlitsch 1998). The land surrounding a wetland is a key component in maintaining viable amphibian populations (Findlay and Houlihan 1997, Marsh and Trenham 2001) because many forage and overwinter on land (Oldfield and Moriarty 1994, Fahrig et al. 1995, Semlitsch and Bodie 2003). Most amphibian adults breed in wetlands, but spend most of the year in upland sites (Oldfield and Moriarty 1994). Metamorphosed juveniles disperse onto land and may migrate distances up to 3 km (de Maynadier and Hunter 1998, Gibbs 1998, Semlitsch and Bodie 2003). Although wetlands and surrounding areas are important for sustaining amphibians, >53% of wetlands in the United States and surrounding forests have been converted to urban or agricultural use (Dahl 1990, Wright et al. 1992).

Agriculture and urbanization reduce and fragment amphibian habitats through the ditching, filling, impervious surfaces, and conversion of wetlands and surrounding habitats into monocultures and development, resulting in lower amphibian richness and diversity (Hecnar 1997, Knutson et al. 1999). Road density, or the ratio of road length to surface area, can cause lower amphibian richness because of runoff from impermeable surfaces, as well as their role as physical barriers to juvenile and adult amphibians (Ashley and Robinson 1996, Houlihan and Findlay 2003, Rubbo and Kiesecker 2005, Eigenbrod et al. 2008). Intense agricultural or urban land use surrounding a wetland can

cause it to be an insularized, artificial island (Knutson et al. 1999). Even though such a wetland is not a true island, isolating land use may prevent connectivity between upland areas and other wetlands, thus making it an artificial island (MacArthur and Wilson 1967). This is particularly true for amphibians that have relatively shorter dispersal distances compared to mammals and birds (Blaustein et al. 1994, Oldfield and Moriarty 1994). This inability to disperse more than just a few kilometers makes gene flow between populations less frequent, especially for isolated populations (Semlitsch 2000) and genetic divergence has been found to be more pronounced with isolation (Reh and Seitz 1990, Hitchings and Beebee 1998). Isolation can make amphibian metapopulations vulnerable to stochastic extinction processes in accordance with the theory of island biogeography (MacArthur and Wilson 1967, Soule 1987, Gibbs 2000). This isolation effect has been observed with amphibian populations in wetlands of the United States due to land use.

The upper Midwest is one region of the United States that has experienced wetland fragmentation and destruction (Brown 1986, Knutson et al. 1999, Keddy 2000), where agriculture is a primary component of the regional economy (Lehtinen et al. 1999, Knutson et al. 2004). One particular wetland type that is experiencing loss in the upper Midwest is peatlands (Mazerolle 2003). Peatlands are freshwater wetlands comprised of decaying organic material (peat; Glaser 1987), and they are considered one of the least disturbed ecosystems (Aaseng and Djupstrom 1992, Desrochers and van Duinen 2006). Within the upper Midwest, Minnesota and Wisconsin have over 3 million ha of peatlands (Aaseng and Djupstrom 1992). Peatlands are considered to be biologically important, including for amphibian populations (Karns 1992, Wright et al. 1992).

Peatlands are biologically important because many wildlife species use peatlands for breeding and foraging sites (Wright et al. 1992). Of the peatland types, sub-boreal peatlands are especially prone to habitat fragmentation due to higher human populations present (Desrochers and van Duinen 2006). A well-defined conservation plan for sub-boreal peatlands in the upper Midwest does not exist due partly to the paucity of knowledge on biodiversity levels present (Calme et al. 2002, Mazerolle 2005, Desrochers and van Duinen 2006), and many are not protected (Ramsar Convention on Wetlands 1996). Studies examining the effect of agricultural and urban land use within buffers surrounding peatlands on amphibian richness and diversity are lacking. Therefore, it may be needed to assess diversity patterns within peatlands so that those with high diversity may be conserved. Amphibian conservation efforts may be improved by identifying the relationships between isolating land use and amphibian populations because peatlands may serve as important habitat refuges for amphibian populations.

In this study, I examined the relationships between isolating land use and amphibian species diversity and richness in a set of sub-boreal peatlands located in the states of Minnesota and Wisconsin in the upper Midwest. In this study, amphibian richness was defined as the number of species present, while amphibian diversity incorporated both the number of species present and the evenness of species present. I examined the relationships at three radii: 500 m, 1000 m, and 2500 m. These spatial scales encompass typical migration and dispersal distances observed in amphibian species found within Minnesota and Wisconsin, with known migration and dispersal distances for species ranging between 60 m to 2500 m (Berven and Grudzien 1990, Oldfield and Moriarty 1994, Lamoureux and Madison 1999, Semlitsch and Bodie 2003,

Lannoo 2005, Forester et al. 2006, Rittenhouse and Semlitsch 2007). Furthermore, these spatial scales have been used in previous studies examining the effects of isolating land use on amphibian populations in Minnesota and Wisconsin (Mensing et al. 1998, Knutson et al. 1999, Lehtinen et al. 1999, Knutson et al. 2004).

I hypothesized that high levels of agricultural and urban land use isolate peatlands via habitat loss and degradation of buffers. More specifically, because agriculture and urban land use reduces gene flow through isolation, the likelihood of “population rescues” from nearby populations would be low, and consequently, species richness and diversity would be low for peatlands that were more isolated. I predicted that species richness and diversity would be greatest for peatlands with low amounts of urban or agricultural land use for all spatial scales. Peatlands with greater isolation were predicted to have lower species richness and diversity.

METHODS

Study area

Seventeen focal peatlands were located in eastern Minnesota and western Wisconsin, USA (Fig. 1, Table 1). These sites were considered to be sub-boreal peatlands due to their location below the 60° N latitude (Swann et al. 2011). All study sites were identified as peatlands in a previous study conducted in 2010 using county soil maps and National Wetland Inventory (NWI) surveys. Only peatlands with access permitted by landowners and permits were included.

Land-use quantification

Determination of relationships between landscape variables and amphibian diversity and species richness was assessed within 500-m, 1000-m, and 2500-m radii extending from a point within each site (Fig. 2; Whited et al. 2000). Landscape context was quantified using ArcMap 10 GIS database (2010 Environmental Systems Research Institute Inc., Redlands, CA). Land use was assessed using 1-m spatial resolution remote-sensing data from 2008 from the National Agriculture Imagery Program (NAIP), United States Department of Agriculture (USDA), and Farm Services Agency (FSA). Within each spatial scale, the land was divided into 1-m² parcels, and these were assigned a land use category. I calculated the proportions of agriculture (cropland), forest, and urban (roads, residential housing, and industrial) within each spatial scale. Isolating land use was defined as the proportion of urban and agricultural (cropland) cover, and was completed for each of the sites at radii of 500 m (Table 2), 1000 m (Table 3), and 2500 m (Table 4). The degree of isolation was calculated as the mean distance from the perimeter of the wetland to the perimeter of the three nearest wetlands that were greater than or equal to 0.5 ha in area (Table 5). This method for calculating isolation was chosen to give a good estimate of how far away each peatland was from nearby wetlands. Even if one wetland was close, it did not necessarily mean it could support amphibian populations. Furthermore, if there was only one close wetland and the others were far away, it would not be accurate to record the peatland as being highly connected. Degree of isolation and wetland area were quantified using ArcMap 10 GIS database. Percent wetland canopy cover was obtained from 2010 data that determined the abundance of canopy species (< 2 m height) using the Minnesota Department of Natural Resources

(2007) relevé method. This method assigned codes for percentage canopy cover of woody plant species >2.0 m in height. Canopy cover was assessed within a 400m² plot that demonstrated the dominant vegetation community at each site and canopy cover codes assigned to each species present.

Surveys

Surveys were conducted four times at each site from April to July in 2011 and 2012. Survey dates were dependent on weather, but for both years, were the first two weeks of each month. During each survey, any amphibians heard calling or seen were recorded and used to calculate amphibian richness (Lehtinen and Galatowitsch 2001, Beja and Alcazar 2003; Shulse et al. 2010). During each survey, aqueous pH within the upper 30 cm of the water column was recorded using a water quality meter (YSI Inc., Yellow Springs, OH) at three within-site locations. I measured pH only in areas with standing water.

For all surveys, sampling was constrained to within 700 m of a point within the peatland. This constraint was necessary because some sites were greater than 300 ha and were bigger than migration distances known for resident species. The distance of 700 m covered the range of most breeding and migration distances stated in previous studies (Oldfield and Moriarty 1994, Semlitsch and Bodie 2003, Lannoo 2005).

Call surveys

Call surveys occurred during May and June according to the North America Amphibian Monitoring Protocol (NAAMP). Call surveys were used to assess species richness. Call surveys began at least 30 min after sunset and were completed by 0100 hr and were conducted under wind speeds less than 20 km hr⁻¹. Surveys were not conducted

in inclement weather or heavy rain. Each site was surveyed at three different points. Selection of points was based on appropriateness for frog calling behavior, with the presence of water being an important factor. Calling at each point was surveyed for 5 minutes, and species heard at each point were identified. Call surveys allow for discernment between *Hyla versicolor* and *Hyla chrysoscelis* which are indistinguishable morphologically (Lehtinen et al. 1999). Calls are similar in the two species but differ in the pulse frequency.

Larval sampling

Larval sampling occurred during the May, June, and July sampling periods and was used to assess species richness and diversity. Larval sampling occurred between 0800 and 2000 hours (Schmutzer et al. 2008). Surveys were not conducted in inclement weather, including heavy rainfall or thunderstorms. For sampling, we dipped a plastic tray (20 x 10 x 5 cm) into the water agitated by walking and then quickly pulled the tray up (Karns 1992). Dips occurred in areas that appeared to be supportive of larval populations to maximize detection ability, with the presence of water an important factor due to larvae requiring water for survival (Lehtinen et al. 1999). All aquatic microhabitats, including surface water and the benthic zone were sampled to avoid bias in species sampling. Ten larvae sampling transects were established at each site with 10 dips in each transect, 1 m apart. Each transect was separated by at least 5 m, although transects varied in length and the distance separating each because of the need of water to conduct the surveys.

Using protocol from the Environmental Protection Agency (2007), larvae collected at each site were placed into a euthanasia chamber (15 x 15 cm plastic

container) that contained a solution of 200 mg/L tricaine methanesulfonate (MS-222, Sigma-Aldrich, St. Louis, MO) buffered with 0.42-1.05 g/L sodium bicarbonate to prevent agitation of the tissues under Institutional Animal Care and Use permit 11-03 (Minnesota State University, Mankato). Larvae were stored in 70% EtOH (Skelly et al. 1999) and subsequently identified. Larvae were kept as voucher specimens to serve as a permanent record. Larvae collected during a particular transect were stored in a container together. Larval *H. versicolor* and *H. chrysoscelis* are morphologically indistinguishable from each other and were grouped together (Skelly et al. 1999). Larvae were collected under Special Permit No. 17027 from the Minnesota Department of Natural Resources and a Scientific Collector's Permit from the Wisconsin Department of Natural Resources.

Statistical analysis

All statistical analyses were conducted using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) for Windows. Data from the four sampling periods within each year were pooled for statistical analysis of species diversity and species richness (Beja and Alcazar 2003; Shulze et al. 2010). Species diversity for each site was calculated using the Shannon-Wiener Index (H' ; Peet 1974). Richness for the calculation of H' was calculated using presence/absence of species assessed from all of the surveys. Evenness was assessed using only data collected from larval surveys. I tested for differences in species richness and species diversity between years using Wilcoxon signed-rank tests ($\alpha=0.05$).

I performed a general linear model (GLM, $\alpha=0.05$) to assess the relationship between species richness and proportion of agricultural and urban land use at each spatial scale. I conducted these GLMs separately for 2011 and 2012 due to major differences in

weather. The year 2011 was considered to be a “wet” year, while 2012 was considered to be a “dry” year. Additional covariates included pH, degree of isolation, proportion wetland canopy cover, proportion of isolating land use, proportion of forested land, and wetland area. All covariates were continuous. Tests for interaction effects between covariates were also performed. The parametric criteria required for a GLM was met for each of the three radii. I began each GLM with a full model using all covariates, but dropped out covariates with the least influence until I reached a reduced model with only those that were significant. Because pH was sampled for both years, a Wilcoxon signed-rank test was performed to assess for differences between the two years.

I assessed the relationship between species diversity and landscape factors at each spatial scale using a generalized linear model with a Poisson distribution due to the failure of the data to meet normality assumptions for a GLM. Covariates were the same as for species richness. The generalized linear models were independently run for 2011 and 2012 data.

Relationships between species richness and each of the covariates were also examined using simple linear regression. This was done for species diversity as well.

RESULTS

Eleven species of amphibians were detected across all sites and years (Table 6). The most common species were *Bufo americanus* (American toad), *Pseudacris crucifer* (spring peeper), *Pseudacris triseriata* (western chorus frog), and *Hyla spp.* (gray treefrog and Cope’s gray treefrog). There was a significant decline ($P < 0.01$) in observed species richness between 2011 and 2012, with a mean of 5.47 (± 0.58 SE) species in 2011 and a

mean of 4.18 (± 0.68 SE) species in 2012 (Fig. 3). There was not a significant difference ($P=0.16$) in species diversity between the two years, with a mean H' of 0.29 (± 0.13 SE) in 2011 and a mean H' of 0.16 (± 0.08 SE) in 2012 (Fig. 4). Site pH was significantly higher ($P<0.001$) in 2012 with a mean of 6.27 (± 0.27 SE) than in 2011 with a mean of 5.94 (± 0.26 SE; Fig. 5).

Isolation was significantly correlated to species richness at each spatial scale ($F=4.60$, $df=16$, $P=0.05$; Table 7) in 2011. Isolation showed a significant negative correlation ($P=0.05$, $r^2=0.24$; Fig. 6) with amphibian richness in 2011, with isolated peatlands having lower species richness. Species richness in 2011 was not correlated with the proportion of isolating land use, area, pH, percent canopy cover, or proportion of forest for all three spatial scales ($P>0.05$). Isolation was also significantly correlated to amphibian richness in 2012 at each spatial scale ($F=9.70$, $df=16$, $P=0.01$; Table 8). Isolation in 2012 showed a significant negative correlation ($P=0.02$, $r^2=0.33$; Fig. 7), with isolated peatlands having lower species richness. Over both years, the most isolated peatlands had an absence of species that have known short dispersal distances such as *H. chrysoscelis* and *H. versicolor*, while a species such as *Rana pipiens* (Northern leopard frog) with a longer dispersal distance was present at one of the more isolated peatlands (Table 9). Besides isolation, canopy cover was significantly correlated with lower species richness in 2012 at all three spatial scales ($F=7.65$, $df=16$, $P<0.05$; Table 8). Canopy cover showed a significant negative correlation with species richness ($P=0.04$, $r^2=0.26$), with peatlands that had a high proportion of canopy cover having lower species richness (Fig. 8). Species richness in 2012 was not significantly correlated with the proportion of isolating land use, area, pH, or proportion of forest for all three spatial

scales ($P>0.05$; Table 8). No interaction effects were detected between the covariates for species diversity or species richness ($P>0.05$). Species diversity was not correlated with the covariates for 2011 ($P>0.05$; Table 10) or 2012 ($P>0.05$; Table 11).

DISCUSSION

My results did not provide evidence that amphibian species diversity and species richness are correlated with isolating land use. This was surprising since numerous studies (*e.g.*, Gibbs 1998, Hels and Buchwald 2001, Knutson et al. 2004, Schmutzer et al. 2008) found that isolating land use does have negative effects on amphibian populations. It is possible, however, that my sites may not have had high enough levels of agricultural and urban land use to show pronounced negative effects. Many of my peatlands had surrounding buffers with less than half of the zone comprised of isolating land use. Other studies found relationships with a degree of isolating land use much greater than those of my sites (Ficetola and De Bernardi 2004, Knutson 2004, Rubbo and Kiesecker 2005, Eigenbrod et al. 2008). These studies also had a greater sampling size (~30-80 wetlands) or had more intensive sampling efforts (*e.g.* 8 night auditory surveys; Eigenbrod et al. 2008) that may also be the reason for the difference in results.

My study also did not support the importance of forest, wetland area, or pH for support of amphibian populations. The lack of correlation between forest cover and amphibian species richness was surprising since other studies noted the importance of forest coverage in adjacent areas for dispersing juveniles and foraging adults (*e.g.*, de Maynadier 1999, Skelly et al. 1999, Houlihan and Findlay 2003).

Peatland area was not predictive of richness or diversity, and this finding supports other studies (Richter and Azous 1995, Semlitsch and Bodie 1998, Snodgrass et al. 2000, Declerck and De Meester 2006). For example, Declerck and De Meester (2006) found that large habitats were not important for promoting amphibian richness, while Semlitsch and Bodie (1998) found that isolation may have a greater impact on amphibians than wetland size.

Although pH has been found to be a factor for amphibian survival (Gosner and Black 1957, Pierce 1985, Karns 1992, Sadinski and Dunson 1992, Grant and Licht 1993), my study did not find any effects on amphibian populations. The difference in pH between the two years may have been attributable to differences in weather. Rainfall was high in 2011, while 2012 was considered to be a drought year. I attributed the decline in species richness in 2012 to the drought (McMenamin et. al. 2008).

Despite isolating land use not showing any correlation with amphibian richness or diversity, my study does support my prediction that greater isolation reduces species richness. This observation supports the island biogeography theory (MacArthur and Wilson 1967) and other studies (*e.g.*, Ficetola and De Bernardi 2004, Parris 2006) from other regions of the world. For example, Parris (2006) discovered a similar pattern for amphibian populations in Australia where isolation by urban roads caused the greatest negative effects on amphibians. Ficetola and De Bernardi (2004) also found a similar relationship in northern Italy, where isolation had a negative impact on resident amphibians. Isolation of a peatland can cause amphibian populations to be less likely to have lower rates of immigration from other areas, causing fewer “population rescues” of an existing population. During stressful situations, such populations may undergo local

extinctions because of the lack of immigration. This is especially true for species that do not disperse long distances. *Hyla chrysoscelis* and *H. versicolor* are two species that tend to disperse less than 800 m from their natal pond (Oldfield and Moriarty 1994, Lanoo 2005) and these two species were typically absent from the most isolated peatlands, with *H. versicolor* only observed at one isolated peatland during a calling survey, and *H. chrysoscelis* completely absent from the isolated peatlands. Although isolation was an important factor, isolation was not always due to habitat loss caused by urbanization or agriculture, but instead by vast tracts of forest, making several of the most isolated peatlands also in areas that had the lowest proportions of agricultural and urban land use in their buffers. Again, this may be support for the island biogeography theory because those isolated peatlands may have been too far away for recolonization (MacArthur and Wilson, 1967). Because some of the isolated peatlands had low proportions of agricultural and urban land use in their buffers, this further supports the idea that isolating land use did not have an effect on species richness or diversity. Even though there was lower species richness at sites with greater isolation, it is likely that the low species richness was also due to scarcity of standing water at these sites, as well as a high proportion of wetland canopy cover that many isolated peatlands had.

Canopy cover caused lower amphibian species richness at all three spatial scales in 2012. These findings support other studies that found that canopy cover over a wetland had negative effects on amphibian populations (*e.g.*, Werner and Glennmeier 1999, Skelly et al. 2002, Halverson et al. 2003, Schiesari 2006). Canopy cover reduces sunlight intensity which causes lower water temperature and lower dissolved oxygen, which impedes larval development (Halverson et al. 2003). These effects may have been

more pronounced in 2012 because of less precipitation and lower water levels caused by the drought.

Even though none of the model variables were predictors of species diversity in 2011 and 2012, I believe that the larval sampling technique could have attributed to this outcome or different catchability between species (Snodgrass et al. 2000). It is possible that some species went undetected because of lower catchability, while others were easier to catch, such as *B. americanus*.

Based on my study, amphibian conservation efforts may be effective by maintaining connectance between peatlands, such as through the use of migration corridors, which has also been suggested by other studies (Gibbs 1998, Semlitsch and Bodie 1998, Ficetola and De Bernardi 2004, Rubbo and Kiesecker 2005). Isolation and canopy cover were correlated with species richness, with greater isolation and canopy cover corresponding to fewer species. This observed relationship between isolation and amphibian richness supports the island biogeography theory. Because of this, there are conservation implications. Minnesota and Wisconsin peatlands may serve as important amphibian refuges, and appropriate conservation actions should be made that take into account isolation and canopy cover. Conservation priority may want to be given to peatlands with low canopy cover and high connectivity to other peatlands. If other peatlands hold true to the island biogeography theory, peatlands that have high connectance should have high species richness. Future research could examine the establishment of habitat corridors and assessing species richness over time. If isolation had an effect, the establishment of habitat corridors should cause an increase in species richness over time.

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FIGURES AND TABLES

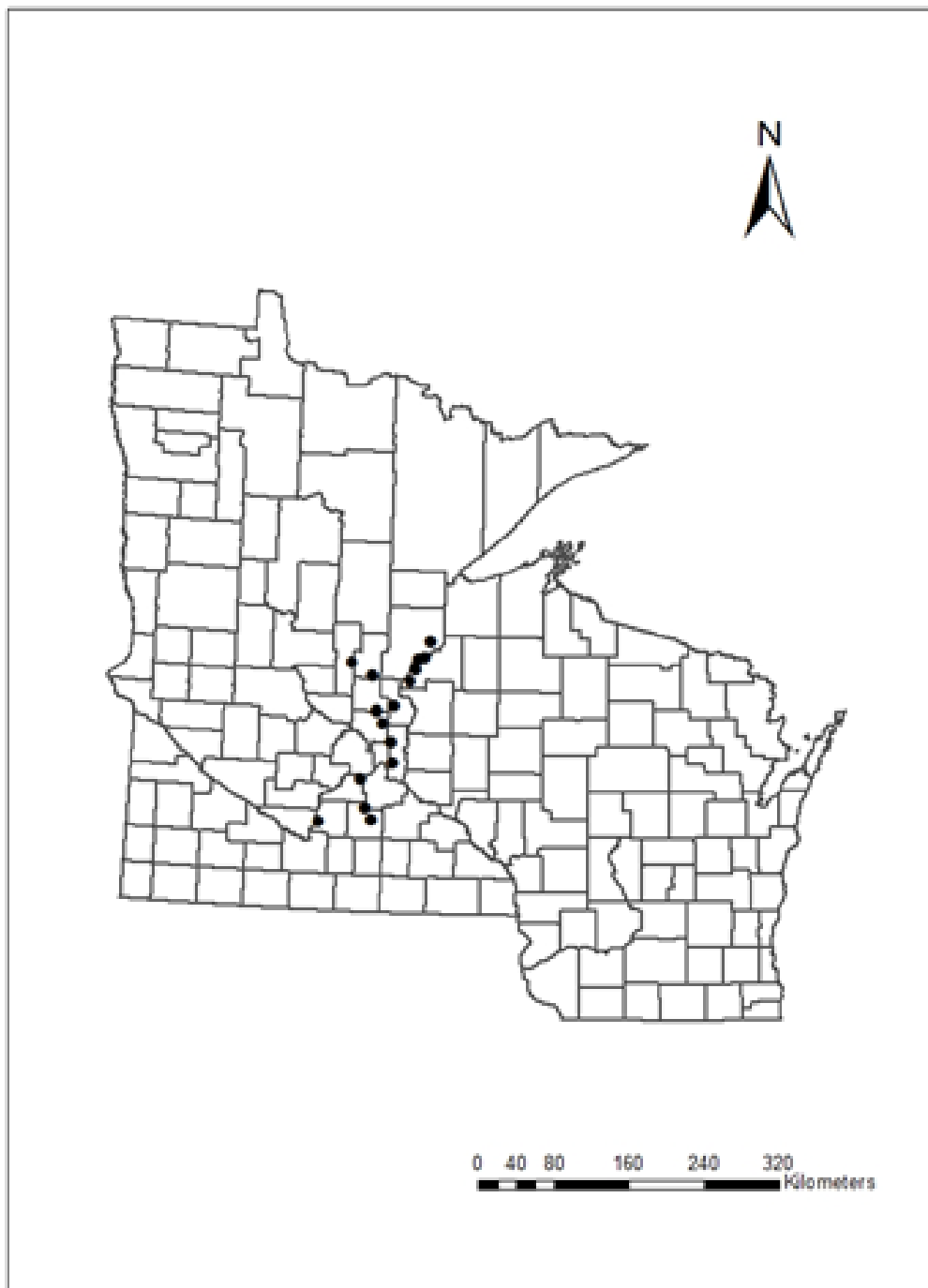


Figure 1. The locations of the seventeen peatlands in Minnesota and Wisconsin. Study sites are represented by a black circle.

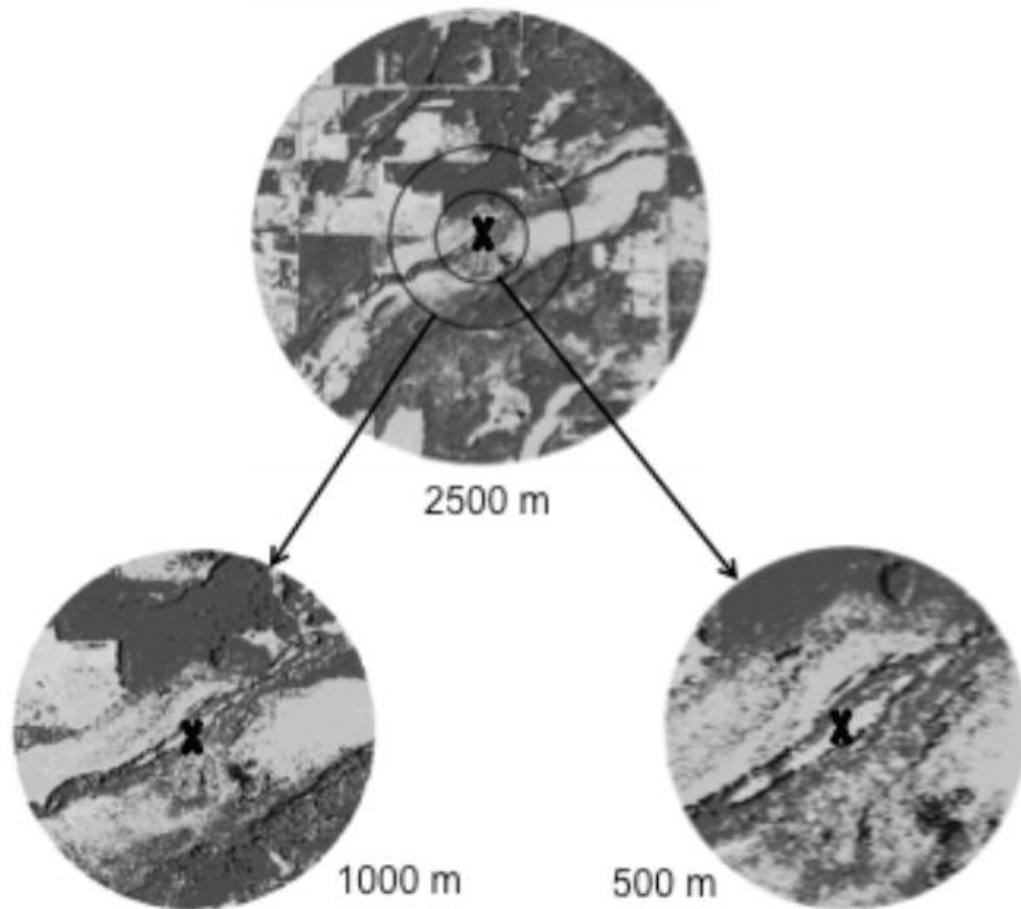


Figure 2. Isolating land use (cropland and urban development) was measured at three spatial scales (500, 1000, and 2500 m radius) for each peatland (n=17). Figure adapted from Whited et al. (2000).

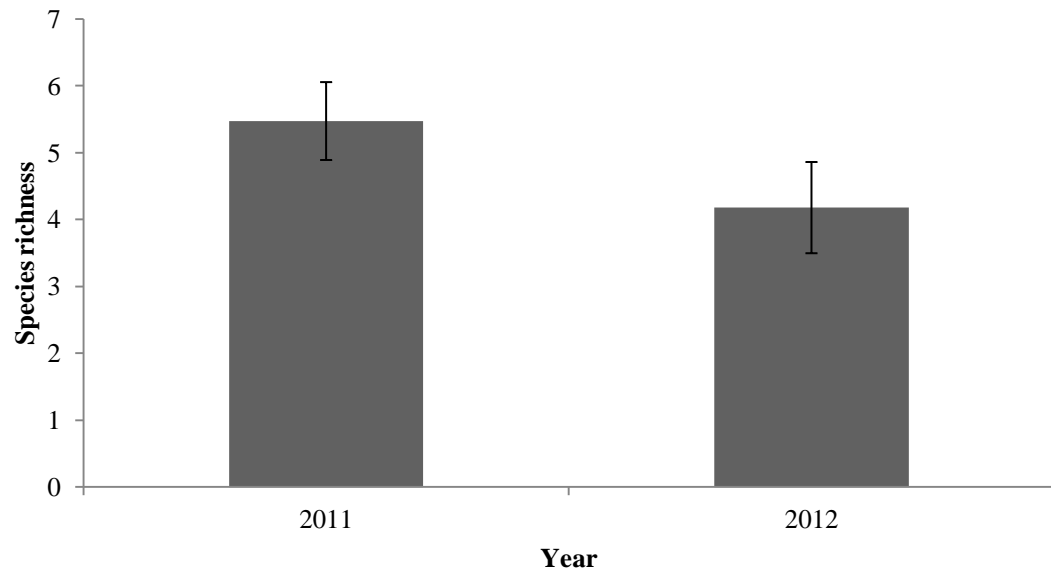


Figure 3. Mean amphibian species richness (\pm SE) of seventeen sub-boreal peatlands in Minnesota and Wisconsin for 2011 and 2012. Mean species richness was 23.66% lower in 2012 ($P < 0.01$), with a mean of 5.47 (± 0.58 SE) species in 2011 and a mean of 4.18 (± 0.68 SE) species in 2012.

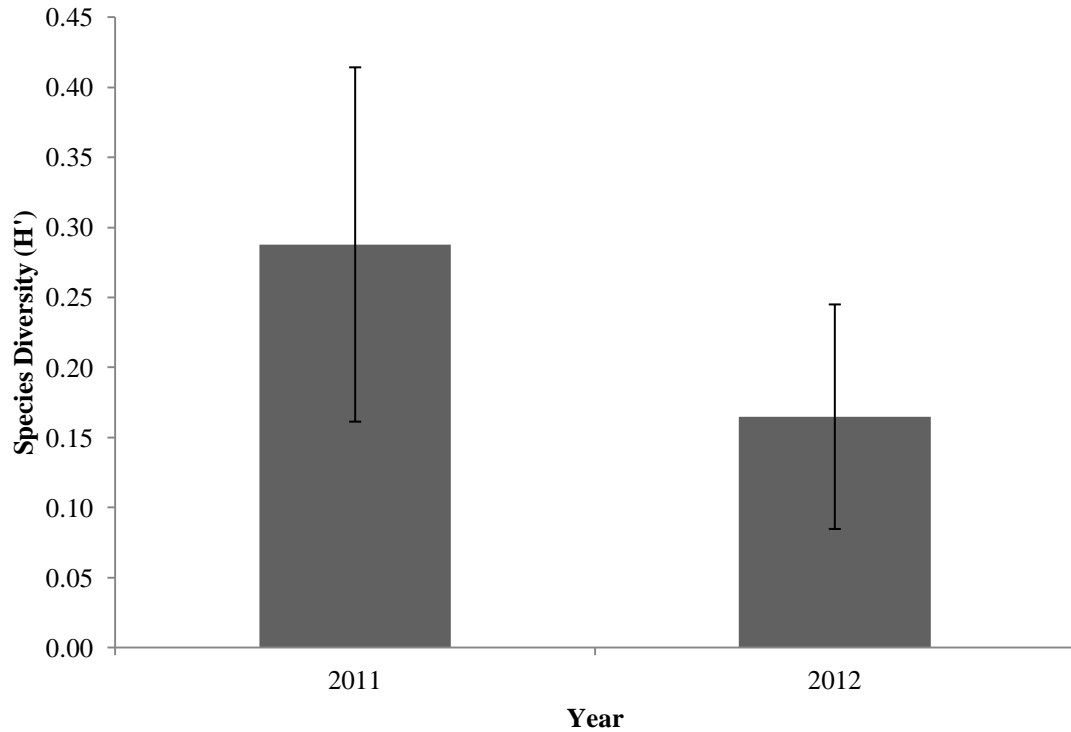


Figure 4. Mean amphibian species diversity (\pm SE) in 2011 and 2012 using the Shannon Wiener Index (H'). Mean species diversity did not significantly differ between the two years ($P > 0.05$), with a mean H' of 0.29 (± 0.13 SE) in 2011 and a mean of 0.16 (± 0.08 SE) in 2012.

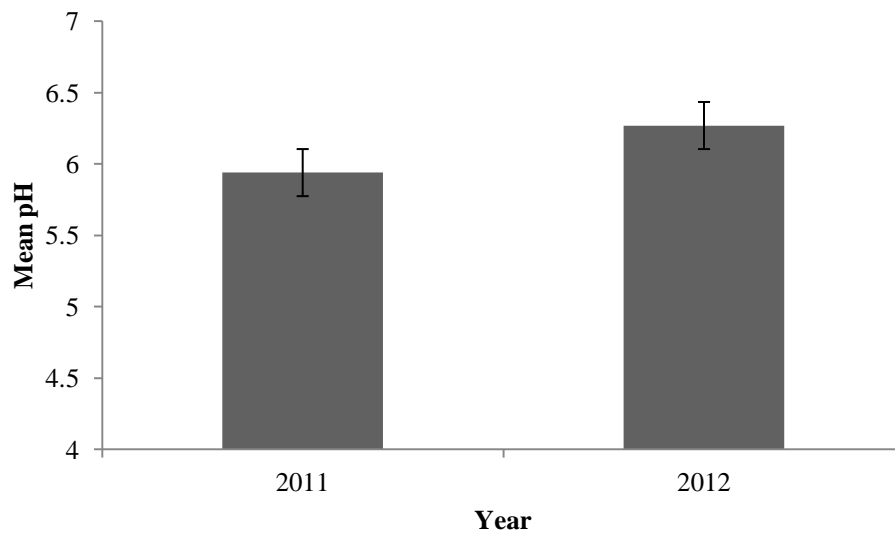


Figure 5. Mean pH (\pm SE) for 2011 and 2012. There was a significant difference ($P < 0.001$) between the two years, with peatlands in 2011 having a mean pH of 5.94 (± 0.26 SE) and peatlands in 2012 having a mean of 6.27 (± 0.27 SE).

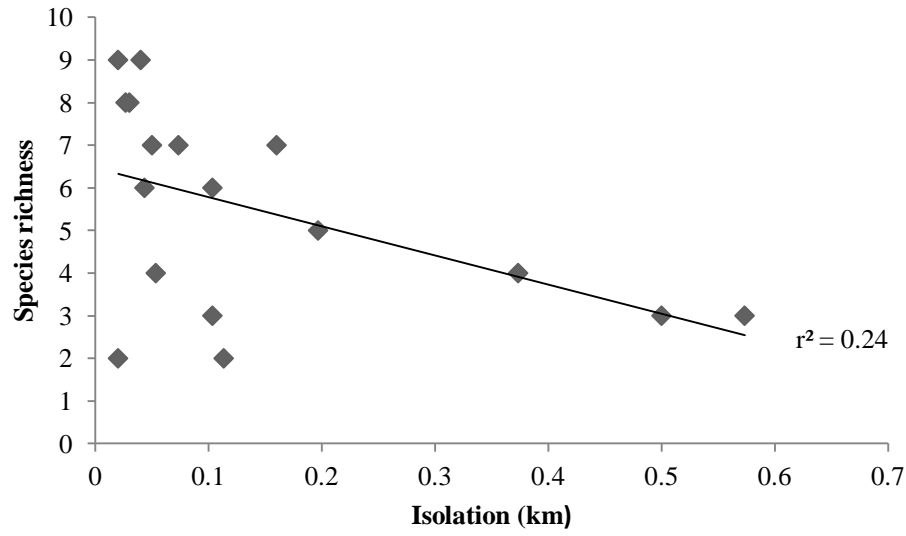


Figure 6. The relationship between isolation and species richness in 2011 ($r^2=0.24$).

Peatlands that were more isolated had significantly lower species richness ($F=4.60$, $df=16$, $P=0.05$).

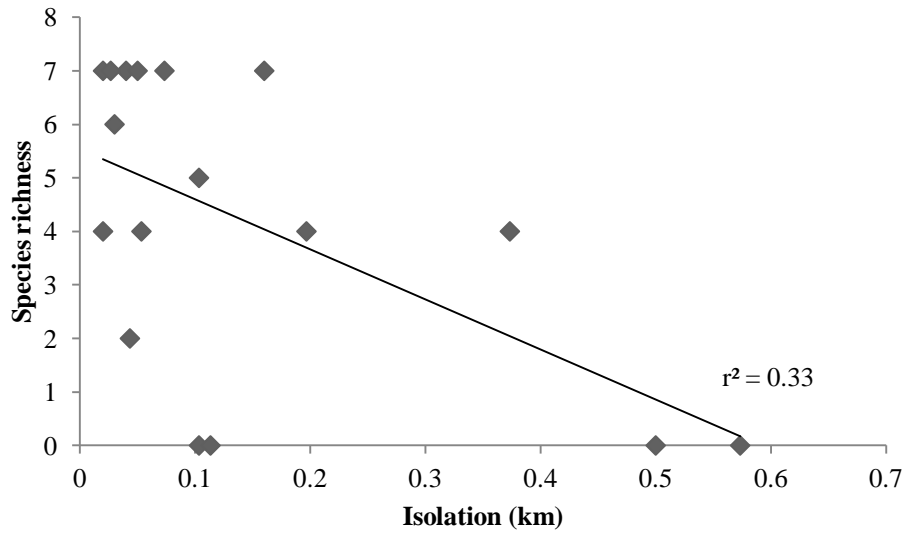


Figure 7. The relationship between species richness in 2012 and isolation ($r^2=0.33$).

Peatlands that were more isolated had significantly lower species richness than less isolated peatlands ($F=7.81$, $df=16$, $P=0.02$).

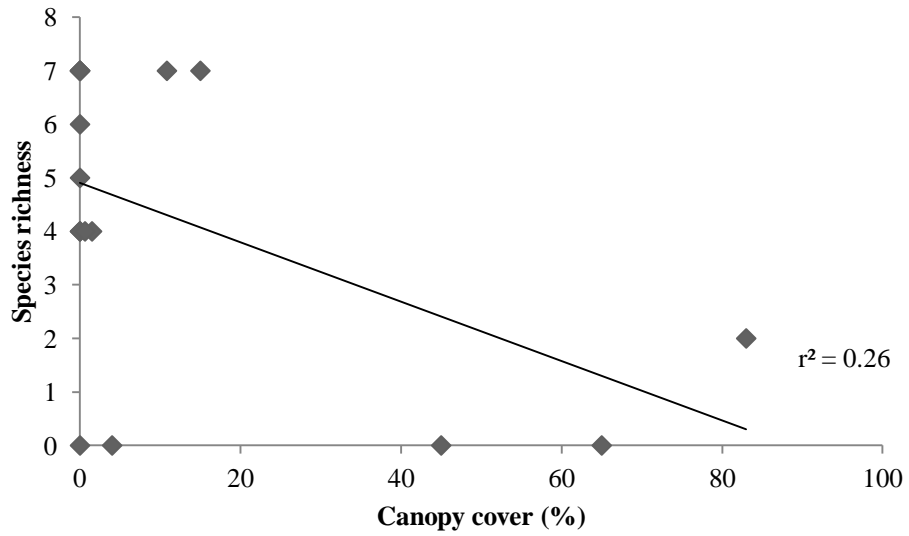


Figure 8. The relationship between species richness in 2012 and canopy cover ($r^2=0.26$).

Peatlands with high canopy cover had significantly lower species richness ($F= 5.17$, $df=16$, $P=0.04$). Two species present under such high canopy conditions included *P. triseriata* and *H. versicolor*.

Table 1. Seventeen focal peatlands with site name, site abbreviation, city and state, and GPS coordinates. The sites were located throughout eastern Minnesota and western Wisconsin.

Site Abbreviation	Site name	City	GPS coordinates
BL	Beckman Lake (CCNHA)	Bethel, MN	45.421784,-93.187014
CA5	Carlos Avery WMA	Forest Lake, MN	45.298349,-93.100912
CC	Cedar Creek NHA	Bethel, MN	45.406551,-93.199129
CR	Cannon River Wilderness Area	Faribault, MN	44.382868,-93.204779
EF	East of Co. Rd. F	Pine City, MN	45.846059,-92.681284
FL	Fish Lake Wildlife Area	Grantsburg, WI	45.714218,-92.751278
JJ	Janet Johnson Memorial WMA	North Branch, MN	45.476604,-92.960542
JN	Jim Nelson Wetland Bank	Mora, MN	45.754771,-93.266697
NP	Norway Point Bottomlands	Grantsburg, WI	45.923643,-92.635998
OT	Ottawa WMA	St. Peter, MN	44.344773,-93.913554
PB	Peat Bog WMA	Faribault, MN	44.484907,-93.298309
RR	Rum River State Forest	Ogilvie, MN	45.871511,-93.565356
SF	St. Croix State Forest	Hinckley, MN	46.102966,-92.492473
SM	Schuneman Marsh	White Bear Lake, MN	45.117,-92.979988
SV	Savage Fen SNA	Savage, MN	44.769473,-93.370514
TS	Tamarack Swamp	Woodbury, MN	44.923329,-92.940738
UC	Union White Cedar	Grantsburg, WI	45.938022,-92.554172

Table 2. The proportion of isolating land use within the 500 m buffer of each peatland.

Peatlands are in order from those with the lowest proportion of isolating land use to those with the greatest proportion of isolating land use in their 500 m buffer.

Site	Proportion of isolating land use within the 500 m buffer
UC	0.00
RR	0.01
FL	0.17
OT	0.17
NP	0.20
EF	0.20
CR	0.21
TS	0.23
CA5	0.23
SV	0.29
CC	0.29
PB	0.34
SM	0.37
SF	0.44
BL	0.45
JJ	0.46
JN	0.60

Table 3. The proportion of isolating land use within the 1000 m buffer of each peatland.

Peatlands are in order from those with the lowest proportion of isolating land use to those with the greatest proportion of isolating land use in their 1000 m buffer.

Site	Proportion of isolating land use within the 1000 m buffer
JN	0.01
PB	0.05
EF	0.16
CA5	0.16
NP	0.18
RR	0.23
SV	0.24
JJ	0.28
FL	0.35
BL	0.36
CR	0.36
OT	0.37
UC	0.38
SF	0.41
SM	0.43
CC	0.46
TS	0.49

Table 4. The proportion of isolating land use within the 2500 m buffer of each peatland.

Peatlands are in order from those with the lowest proportion of isolating land use to those with the greatest proportion of isolating land use in their 2500 m buffer.

Site	Proportion of isolating land use within the 2500 m buffer
JN	0.02
CA5	0.12
PB	0.13
EF	0.13
NP	0.16
RR	0.16
CR	0.31
JJ	0.35
SF	0.35
BL	0.36
SM	0.36
FL	0.37
SV	0.37
UC	0.39
CC	0.50
TS	0.52
OT	0.52

Table 5. The degree of isolation (km) of each peatland as defined as the mean distance from the perimeter of the peatland to the nearest three wetlands greater than or equal to 0.5 ha in size. Peatlands are in order from those with the lowest degree of isolation to those with the greatest degree of isolation.

Site	Isolation (km)
EF	0.02
TS	0.02
FL	0.03
CA5	0.03
BL	0.04
UC	0.04
CC	0.05
SV	0.05
JN	0.07
CR	0.10
JJ	0.10
SF	0.11
SM	0.16
PB	0.20
RR	0.37
NP	0.50
OT	0.57

Table 6. Amphibian species observed in all surveys in seventeen sub-boreal peatlands in Minnesota and Wisconsin during the period from April to July in 2011 and 2012.

Number of sites that each species was observed in 2011 and 2012 is shown.

Species Name	Common Name	No. of Sites 2011	No. of Sites 2012
<i>Ambystoma laterale</i>	Blue-spotted salamander	1	1
<i>Bufo americanus</i>	American toad	14	10
<i>Hyla chrysoscelis</i>	Cope's gray treefrog	5	4
<i>Hyla versicolor</i>	Gray treefrog	14	13
<i>Pseudacris crucifer</i>	Spring peeper	11	9
<i>Pseudacris triseriata</i>	Western chorus frog	15	13
<i>Rana catesbeiana</i>	American bullfrog	1	0
<i>Rana clamitans</i>	Green frog	9	6
<i>Rana pipiens</i>	Leopard frog	10	8
<i>Rana septentrionalis</i>	Mink frog	2	2
<i>Rana sylvatica</i>	Wood frog	9	5

Table 7. Summary of GLM analysis for amphibian species richness during April-July of 2011 in seventeen sub-boreal peatlands of Minnesota and Wisconsin at three spatial scales (500, 1000, and 2500 m). The initial model included all covariates, but non-significant covariates were eliminated until only the significant ones remained. Isolation was correlated with species richness at all three spatial scales ($F=4.60$, $df=16$, $P=0.05$, $r^2=0.24$). There were no significant interactions among covariates ($P>0.05$).

Variable	500 m				1000 m				2500 m			
	Df	F	P	r ²	Df	F	P	r ²	Df	F	P	r ²
Corrected Model	16	4.60	0.05	0.24	16	4.60	0.05	0.24	16	4.60	0.05	0.24
Isolation	16	4.60	0.05		16	4.60	0.05		16	4.60	0.05	

Table 8. Summary of GLM analysis for amphibian species richness during April-July of 2012. The initial model included all covariates, but non-significant ones were eliminated until only the significant covariates remained. Isolation ($F=9.70$, $df=16$, $P=0.01$) and proportion canopy cover ($F=7.65$, $df=16$, $P=0.02$) were significantly correlated ($r^2=0.56$) with species richness for all three spatial scales. There were no significant interaction effects between any of the covariates ($P>0.05$).

Variable	500 m				1000 m				2500 m			
	Df	F	<i>P</i>	r^2	Df	F	<i>P</i>	r^2	Df	F	<i>P</i>	r^2
Corrected Model	16	8.94	0.003	0.56	16	8.94	0.003	0.56	16	8.94	0.003	0.56
Isolation	16	9.70	0.01		16	9.70	0.01		16	9.70	0.01	
Proportion canopy cover	16	7.65	0.02		16	7.65	0.02		16	7.65	0.02	

Table 9. Summary of species present at each site with known mean dispersal distances and degree of isolation for each site. Species that were present at each site is indicated by an “X.”

Species	Mean Dispersal Distance (m)	Sites																
		EF	TS	FL	CA5	BL	UC	CC	SV	JN	CR	JJ	SF	SM	PB	RR	NP	OT
Bullfrog	3200															X		
Leopard Frog	2900	X	X	X	X	X		X		X	X	X		X				X
Green Frog	1810	X		X	X	X	X	X	X	X		X						
Wood Frog	1600	X		X	X	X	X	X		X			X	X		X		
American Toad	1000	X	X	X	X	X	X	X	X	X		X		X	X		X	X
Gray Treefrog	800	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Cope's Gray Treefrog	613	X		X	X	X		X						X	X			
Isolation (m)		20	20	27	30	40	43	50	53	73	103	103	113	160	197	373	500	573

Table 10. Summary of generalized linear model analysis for amphibian species diversity (Shannon Wiener Index; H') during April-July of 2011. None of the variables were correlated with amphibian species diversity ($P > 0.05$)

Variable	<i>500 m</i>			<i>1000 m</i>			<i>2500 m</i>		
	Df	Wald Chi-Square	<i>P</i>	Df	Wald Chi-Square	<i>P</i>	Df	Wald Chi-Square	<i>P</i>
Area	16	2.986	0.084	16	2.700	0.100	16	2.001	0.157
Isolation	16	0.053	0.819	16	0.030	0.863	16	0.165	0.685
pH	16	0.184	0.668	16	0.986	0.321	16	0.933	0.334
Isolating land use	16	0.167	0.683	16	0.182	0.670	16	0.264	0.607
Proportion forest	16	0.652	0.419	16	0.810	0.368	16	0.326	0.568
Canopy cover	16	0.658	0.417	16	0.343	0.558	16	0.241	0.568

Table 11. Summary of generalized linear model analysis for amphibian species diversity (Shannon Wiener Index; H') during April-July of 2012. None of the variables were correlated with amphibian species diversity ($P > 0.05$).

Variable	<i>500 m</i>			<i>1000 m</i>			<i>2500 m</i>		
	Df	Wald Chi-Square	<i>P</i>	Df	Wald Chi-Square	<i>P</i>	Df	Wald Chi-Square	<i>P</i>
Area	16	1.427	0.232	16	1.461	0.200	16	1.008	0.315
Isolation	16	0.575	0.232	16	0.59	0.442	16	1.427	0.232
pH	16	0.533	0.465	16	1.714	0.190	16	1.566	0.211
Isolating land use	16	0.005	0.942	16	0.171	0.679	16	0.155	0.693
Proportion forest	16	0.652	0.419	16	2.366	0.124	16	1.818	0.178
Proportion canopy cover	16	0.027	0.868	16	0.176	0.675	16	0.272	0.602