*Original Contribution*

# Land Use and Wetland Spatial Position Jointly Determine Amphibian Parasite Communities

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**Abstract:** Land use change is one of the most commonly cited contributing factors to infectious disease emergence, yet the mechanisms responsible for such changes and the spatial scales at which they operate are rarely identified. The distributions of parasites with complex life cycles depend on interactions between multiple host species, suggesting the net effects of land use on infection patterns may be difficult to predict a priori. Here, we used an information-theoretic approach to evaluate the importance of land use and spatial scale (local, watershed, and regional) in determining the presence and abundance of multi-host trematodes of amphibians. Among 40 wetlands and 160 hosts sampled, trematode abundance, species richness, and the presence and abundance of pathogenic species were strongly influenced by variables at the watershed and regional scales. Based on model averaging results, overall parasite richness and abundance were higher in forested wetlands than in agricultural areas; however, this pattern was influenced by a wetland's proximity to the Mississippi Flyway at the regional scale. These patterns likely reflect the activity of trematode definitive hosts, such as mammals and especially birds, such that infections decreased with increasing distance from the Mississippi River. Interestingly, despite lower mean infections, agricultural wetlands had higher variances and maximum infections. At the wetland scale, phosphorus concentrations and the abundances of intermediate hosts, such as snails and larval amphibians, positively affected parasite distributions. Taken together, these results contribute to our understanding of how altered landscapes affect parasite communities and inform further research on the environmental drivers of amphibian parasite infections.

**Keywords:** spatial epidemiology, ribeiroia, echinostoma, amphibian decline, conservation, land use change

## INTRODUCTION

Understanding the environmental drivers of parasite distributions and their consequences for disease occurrence is

the focus of the emerging field of spatial epidemiology (Ostfeld et al. 2005; Joly et al. 2006; Swaddle and Calos 2008). It is particularly challenging to determine which factors influence the spatial distribution of parasites with complex life cycles, which require the presence of both intermediate and definitive hosts to sustain parasite transmission (Thieltges and Reise 2007). Hosts and vectors in these life cycles exhibit different geographic distributions and may be influenced by land use patterns through different mechanisms or to differing extents (Smith 2001; Fredensborg et al. 2006; Byers et al. 2008). Despite such challenges, knowledge of the drivers of parasite distributions and the scales at which they operate is critical to determine disease risk for humans and wildlife (Daszak et al. 2001; Ostfeld et al. 2005).

The multi-host life cycles of trematode parasites and their interactions with hosts of widely differing life history traits make them an excellent system in which to examine the influence of spatial scale on distribution patterns (Byers et al. 2008). Trematodes use molluscs as first intermediate hosts, invertebrates, and vertebrates as second intermediate hosts, and various vertebrates as definitive hosts. Most intermediate hosts are sessile or move only short distances (Latham and Poulin 2003), such that local processes may have a strong influence on their infection levels. Definitive hosts such as birds and mammals, in contrast, are more mobile and have the potential to impose large-scale spatial patterns in parasite distribution and abundance (Bustnes and Galaktionov 1999; Fredensborg et al. 2006; Smith 2007). For example, the distribution of the avian trematodes responsible for cercarial dermatitis closely follows bird migration flyways (Jarcho and van Burkalow 1952; Chu 1958). Understanding the drivers of parasite distributions requires examination of the spatial scales used by each host group and each parasite life stage, as well as interactions with the abiotic environment and with non-host species in the community.

Changing land use patterns can have multiple, potentially opposing effects on parasite life cycles and transmission. For example, agricultural or urban development can increase nutrient enrichment in aquatic systems, triggering enhanced algal productivity with concomitant increases in the abundance of first intermediate host snails and their potential to support infections (Johnson and Chase 2004; Skelly et al. 2006; Johnson et al. 2007). Pesticide exposure from agricultural runoff can also impair amphibian immune function and parasite avoidance behavior, indirectly leading to an increase in infection and pathology (Kiesecker 2002; Christin et al. 2003; Gendron et al. 2003; Rohr et al. 2008). However, agricultural activities are sometimes associated with lower infections in amphibians (Koprivnikar et al. 2006, 2007). Pesticide exposure can decrease the life span or infectivity of free-living transmission stages (Pietrock and Marcogliese 2003) while agricultural activities may degrade terrestrial habitats and reduce definitive host activity (King et al. 2007, 2010). For example, herbicide use and wetland drainage has been linked to a reduced abundance of waterfowl (Jarcho and van Burkalow 1952; Lemly et al. 2000) while deforestation can similarly reduce populations of mammal definitive hosts (Schell 1985; Lomolino and Perault 2000; Craig and Craig 2005). The conflicting effects of environmental change on the abundance and activity of different hosts in trematode life cycles challenges our ability to predict the net consequences of land use transformations on parasite infections.

To reconcile the complex and sometimes opposing effects of how land use change influences multi-host parasite infections, we examined abiotic and biotic drivers of infection in amphibians across multiple spatial extents, including local (wetland community composition and physicochemical characteristics), watershed (agricultural vs. forested watersheds), and regional (geographic proximity to a major bird flyway). We focused in particular on trematodes known to cause pathology in amphibians, such as *Ribeiroia ondatrae*, which causes severe malformations and elevated mortality (Johnson et al. 1999, 2001), and the echinostomes (*Echinostoma trivolvis*, *Echinostoma revolutum*, and *Echinoparyphium* spp.), which can reduce the survival and growth of amphibian larvae (Fried et al. 1997; Schotthoefer et al. 2003; Holland et al. 2007). The life cycles of both parasite groups involve pulmonate snails as first intermediate hosts, amphibian larvae or fish as second intermediate hosts, and amphibian-eating birds or mammals as definitive hosts, with the notable difference that snails can also serve as second intermediate hosts for echinostomes (Dillon 2000; Johnson and McKenzie 2008). We predicted that agricultural land-use and wetland spatial position would influence the abundance and distribution of intermediate and definitive hosts and, by extension, patterns of trematode infection in amphibians. We constructed and evaluated specific hypotheses incorporating variables from different spatial scales using an information-theoretic approach in combination with model averaging. Considering widespread patterns of land-use change, emerging diseases, and amphibian population declines (Daszak et al. 2003; Stuart et al. 2004), this study has the

potential to contribute to our understanding of how altered landscapes affect trematode communities and host disease risk.

## MATERIALS AND METHODS

### Wetland Selection

To evaluate the effects of land use and spatial position on helminth communities in amphibians, we selected 40 wetlands along two transects: a predominantly agricultural transect located in west-central Wisconsin, USA (22 wetlands) and a forested transect located in northwestern Wisconsin, USA (18 wetlands). The agricultural transect sites included mostly artificial ponds on private land constructed for irrigation and livestock, whereas the forested transect was largely on federal and state public lands and included a mixture of natural ponds, small lakes, beaver ponds, and artificial roadside ponds. The agricultural transect was positioned within the Eastern Temperate Forest ecoregion while the forested transect was within the Northern Forest ecoregion (Omernik et al. 2000), but neither transect crossed an ecoregion boundary suggesting that there were few systematic differences within the transects. However, for this study and for many agricultural areas in the Upper Midwest generally, land use (e.g., forested vs. agricultural) and spatial position (e.g., transect position) are inextricably linked.

To sample sites of varying distances from the high avian activity associated with the Mississippi Flyway (Grettenberger 1991; Best et al. 1995; Knutson et al. 1996), we positioned the western-most point of each transect at the center the Mississippi Flyway (i.e., the Mississippi River) and the length of each transect, running eastward, perpendicular to the flyway. This allowed us to use distance to the Mississippi River, which varied from 11 to 171 km along the transects (Fig. 1), as a surrogate for distance to the Mississippi Flyway. The Mississippi River Flyway is one of the predominant bird migratory routes through North America and annually supports the movement of several million birds representing >300 species (Lincoln et al. 1998). Although, the specific boundaries of bird flyways are imprecise and can vary among species, populations, and years (Reed et al. 2003), we assumed that bird densities and diversity would be greatest close to the Mississippi River with subsequent decreases with greater distances eastward. This pattern is supported for a number of species (Lincoln



**Figure 1.** Map of Wisconsin, USA showing wetlands sampled during summer 2006. The *top line* represents the forested transect and the *bottom line* represents the agricultural transect. The Mississippi River is located along the western border of Wisconsin.

et al. 1998), including dabbling ducks, which can show 70% declines in abundance over ∼100 km distance from the Mississippi (Bellrose 1968). Because bird density and diversity can be challenging to estimate reliably (e.g., Hechinger and Lafferty 2005), particularly using snapshot surveys across a large geographic area that contains numerous cryptic species, our use of proximity to the Mississippi River as a surrogate for bird density provided a more time-integrated approach to estimating patterns of avian activity. Correspondingly, migratory flyways have become an important tool in understanding several other diseases transmitted by waterfowl, such as avian schistosomes and West Nile virus (Jarcho and van Burkalow 1952; Reed et al. 2003; Jourdain et al. 2007).

To reduce the influence of spatial autocorrelation and increase the independence of sampled wetlands, we divided transects into 9.6-km wide sections and used topographic maps to locate wetlands in each section. Selected wetlands had an average surface area of 11.4 ha (range: 0.05–72.6 ha) and were within 1 km of an accessible road or entry point. While this introduced some measure of non-randomness, it was necessary to obtain landowner permission and we employed the same selection criteria in both transects. We further divided our study area into

1:24,000 spatial resolution watersheds (http://dnr.wi.gov/maps/gis/datahydro.html) (average watershed size = 629 km$^2$) and used the National Land Cover Data land use layer in GIS ArcInfo to determine the percentage of area covered by row-crops, forested land, wetlands, and open water surrounding each of our study sites. The watersheds along the agricultural transect averaged 41.5% agricultural area and 28.9% forested area, whereas watersheds along the forested transect averaged 0.6% agricultural and 69.2% forested area (Table 1).

## Wetland Surveys

We sampled wetlands from June 26 to 30 Aug 2006. To minimize possible effects of seasonal shifts in community composition, we alternated sampling visits between the agricultural and forested transects. At each wetland, we estimated the presence and abundance of amphibians during visual-encounter-surveys conducted around the perimeter of the wetland, walking at a constant pace and counting all animals in front of the observer and within 1 m on either side (Heyer et al. 1994). Then, we set up four transects around the wetland's perimeter and conducted four standardized dipnet sweeps (1.4 mm mesh size, 2600 cm$^2$ opening) every 15 m along each transect, for a total of 16 sweeps. For each sample, we pulled the dipnet quickly through the water column for a distance of 1 m perpendicular to the shore and counted the number of snails and larval amphibians. To minimize measurement bias during visual-encounter-surveys and

netsweeps, the same observer (RBH) sampled all 40 sites, limited sampling to the time period of 4 h after sunrise to 4 h before sunset, and did not sample during precipitation events. We collected water samples and measured conductivity ($\mu$S cm$^{-1}$), total nitrogen content ($\mu$g L$^{-1}$), and total phosphorus content ($\mu$g l$^{-1}$) using standardized methods (lter.limnology.wisc.edu/protocols.html).

## Parasite Evaluation

To evaluate the patterns of parasite infection in amphibian hosts, we collected five metamorphic green frogs (Lithobates [=Rana] clamitans) from each site for necropsy. In small wetlands such as those studied here, larval helminth infection prevalence (percentage of infected frogs) often increases rapidly with infection abundance (number of parasites per frog), such that a small number of animals can provide a reliable indicator of parasite presence and species richness while minimizing the destructive sampling of amphibians (see Fig. 3). We evaluated the uncertainties of this approach using rarefaction curves and bootstrapping analysis (see below). When L. clamitans were not available in sufficient numbers, we captured Anaxyrus [=Bufo] americanus or L. pipiens as surrogate hosts ($n$ = 5 wetlands). However, because amphibian species vary in development time and susceptibility to trematode infection, we also performed all analyses of trematode abundance and richness without these wetlands. To quantify helminth infections, we used a stereo-dissecting microscope to thoroughly examine the skin (externally and internally), major

**Table 1.** Descriptive data for wetlands surveyed in northern and central Wisconsin.

| Variable | Agricultural transect ($n$ = 22) | Forested transect ($n$ = 18) | $P$ value |
|---|---|---|---|
| Agricultural area (%) | 41.47 ± 1.7 | 0.61 ± 0.13 | <0.0001 |
| Forested area (%) | 28.85 ± 1.9 | 69.22 ± 1.6 | <0.0001 |
| Pond area (m$^2$) | 5853 ± 1392 | 18,512 ± 5175 | 0.02 |
| Distance to Mississippi River (km) | 102 ± 11.7 | 67.9 ± 8.2 | NS |
| Total N ($\mu$g l$^{-1}$) | 2794 ± 483 | 1234 ± 295 | 0.002 |
| Total P ($\mu$g l$^{-1}$) | 339 ± 82.6 | 42.6 ± 9.9 | <0.0001 |
| Conductivity ($\mu$S cm$^{-1}$) | 253 ± 110.7 | 92.9 ± 30.9 | 0.002 |
| Snail density (no. sweep$^{-1}$) | 2.83 ± 1.3 | 0.653 ± 0.21 | 0.04 |
| Tadpole density (no. sweep$^{-1}$) | 0.63 ± 0.49 | 0.063 ± 0.02 | NS |
| Frog density (no. min$^{-1}$) | 5.56 ± 1.9 | 1.67 ± 0.34 | NS |
| Snail species richness | 2.1 ± 0.31 | 2.1 ± 0.38 | NS |

Listed are the mean values (±1 SE) of each variable for wetlands along the agricultural and forested transects. $P$ values represent the significance values for independent samples $t$ tests comparing the log$_{10}$-transformed values (or arcsine-square root-transformed values in the case of proportions) of each variable on agricultural versus forested wetlands (equal variances not assumed).

organ systems, and digestive tract of each animal. We isolated and excysted all parasite metacercariae and examined them alive to facilitate identification using a compound microscope with Nomarksi optics. We counted and identified to species (*R. ondatrae*), genus (Alaria, Fibricola, Apharyngostrigea and Haematoloechus), or family (unidentified Strigeidae and echinostomes [a possible mixture of *Echinostoma* spp. and *Echinoparaphyium* spp.]) all larval and adult trematodes following Yamaguti (1975), Schell (1985), and Sutherland (2005). We isolated metacercariae of echinostome trematodes from the kidneys of infected frogs and identified by characteristic collar spines.

Because, subsampling can limit the ability to detect all the species in a community, we used the Species Richness Estimators Eco-Tool (Russell 2006), which implements the techniques described in Colwell and Coddington (1994) and Colwell et al. (2004), to assess the reliability of our parasite richness estimates. Specifically, we compared our richness observations to bias-corrected chao2 estimates of richness and generated sample-based rarefaction curves for each site (Walther and Morand 1998; Zelmer and Esch 1999; Dove and Cribb 2006). Chao2 uses presence–absence data, specifically the number of species that occur in only 1 sample and the number that occur in exactly 2 samples, to estimate the number of species present at a site but unaccounted for in sampling. To further explore the influence of host sample size on parasite detection, we used data from an intensively studied wetland located in close geographic proximity (Eden Prairie, Minnesota, USA). Because, we exhaustively sampled parasite infections from 78 metamorphic northern leopard frogs (Lithobates [=Rana] pipiens) at this site, it provided a unique opportunity to compare the influence of subsampling on our estimates of parasite richness and abundance (see Walter and Moore 2005). We used the Species Richness Estimators Eco-Tool to create a sample-based rarefaction curve of larval trematode species richness that compared the chao2 richness estimates derived from 5 hosts against those from more hosts. Similarly, to determine how host sample size affected variance around the estimates of *R. ondatrae* and echinostome abundance, we used bootstrapping analysis without replacement (5,000 iterations) to examine how changes in sample size influenced the reliability of subsampling approaches relative to calculations from the complete data set.

## Model Construction and Analysis

We developed models to predict five response variables: larval trematode species richness (cumulative estimate from all necropsied frogs at a site), total larval trematode abundance (average from a site), abundance of echinostomes (averaged from a site), abundance of *R. ondatrae* infection (averaged from a site), and the presence of *R. ondatrae* (yes or no for each site). We did not develop a model for echinostome presence because echinostome parasites were encountered at all but three sampled wetlands. We focused our analyses on *R. ondatrae* and the echinostomes because infection by these trematodes has been linked to increased mortality of tadpoles as well as physiological and developmental pathologies (Johnson et al. 1999, 2001; Holland et al. 2007). The remaining trematode species were either not sufficiently abundant to warrant individual analysis or not known to cause pathology in amphibians (although some post hoc analyses were performed).

We used an information-theoretic approach to select the most appropriate models for predicting our response variables. The information-theoretic approach relies on the construction of well-considered candidate models based on previous literature (Burnham and Anderson 2002; Marchetti et al. 2004). Our candidate models contained land use (percentage forested area), spatial position (distance to the Mississippi Flyway), intermediate host abundances (i.e., amphibians and snails), nutrient concentrations, and conductivity levels (Table 2). In agriculturally dominated landscapes, we predicted nutrient runoff to increase algal productivity and snail biomass while conductivity would be associated with increased $Ca^{2+}$ concentrations, which often positively affect snail distributions (e.g., Lewis and Magnuson 2000; Johnson and Chase 2004; Skelly et al. 2006; Johnson et al. 2007; Rohr et al. 2008) (although conductivity can also serve as another proxy for total productivity); at the same time, however, we predicted that agricultural area would reduce the availability of avian and mammalian habitat, resulting in lower definitive host activity and reduced trematode transmission (Lafferty 1997; Hechinger and Lafferty 2005). As a result of these competing factors, we predicted that trematode richness and abundance would be more variable in agriculturally dominated landscapes compared to more natural landscapes. We further predicted that wetlands closer to the Mississippi Flyway would have increased trematode infections due to elevated avian definitive host activity (Grettenberger 1991; Best et al. 1995; Knutson et al. 1996).

We classified our predictor variables into one of three categories: definitive host abundance (DH), intermediate host abundance (IH), or physicochemistry (PC). Our

**Table 2.**  Candidate models developed to predict trematode response variables.

| Model | Category | A priori | Definitive hosts | | Intermediate hosts | | | | Physicochemistry | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pond area | Proximity to Mississippi | Forested area | Snail density | Snail richness | Tadpole density | Frog density | Total phosphorus | Conductivity |
| 1 | Global | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | SV | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | SV | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | SV | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 5 | SV | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 6 | SV | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 7 | SV | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 8 | SV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 9 | SV | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 10 | DH | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | IH | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 12 | IH | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 13 | IH | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 14 | PC | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 15 | DH + IH | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 16 | DH + IH | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 17 | DH + IH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
| 18 | DH + IH | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 |
| 19 | DH + IH | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 20 | DH + PC | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| 21 | DH + PC | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| 22 | DH + PC | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| 23 | DH + PC | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 24 | DH + PC | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| 25 | IH + PC | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| 26 | IH + PC | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| 27 | IH + PC | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| 28 | IH + PC | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| 29 | IH + PC | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 |

The first model listed is the global model, which includes all possible independent variables, while the remaining models represent single–variable models (SV) or combinations of definitive host factors (DH), intermediate host factors (IH), and physicochemical factors (PC). All models included wetland surface area as an a priori obvious variable. A "1" indicates that a given predictor was included in the model. No interaction terms were included in the analysis.

specific predictor variables included distance to the Mississippi River and percentage of forestland within the watershed (DH), snail density (individuals netsweep$^{-1}$), snail species richness, tadpole density (individuals netsweep$^{-1}$), and frog density (individuals min$^{-1}$) (IH), and conductivity and phosphorus content (PC). We transformed continuous data with the logarithmic function and proportional data with the arc-sin square root function. While land use (forested vs. agricultural) and transect identity (north vs. central) were intentionally linked in this study (Spearman $\rho = 0.86$, $P < 0.001$), we used land use as the predictor because it represents the expected biological mechanism through which observed differences between transects likely arise. Because, the percentages of agricultural and forested land were strongly collinear ($r = -0.97$), however, we included only the percentage of forested area in our analyses. We also excluded total nitrogen content ($\mu g\ l^{-1}$) because it was highly correlated with total phosphorus content ($\mu g\ l^{-1}$) ($r = 0.811$). No other selected predictors were collinear (all pairwise correlations $r < 0.7$). For each response variable, we created 29 models based on our hypotheses, each of which was categorized according to what predictor variable(s) were included (Table 2). The candidate model categories were as follows: DH, IH, PC, DH + IH, DH + PC, IH + PC. In addition, we included single-variable models (SV), each of which contained two parameters (representing the intercept and a coefficient for the predictor variable of interest), and a global model that included all available predictor variables (Table 2). We included pond area as an ''a priori obvious'' variable in each model as we expected it to associate with trematode presence, abundance, and richness.

We used multiple linear- and logistic regression analyses to confirm that the global model provided a good fit to the data before proceeding with the information-theoretic approach (Burnham and Anderson 2002). We used a small sample size adjustment to Akaike's Information Criterion (AIC$_C$) to rank each candidate model, where a lower AIC$_C$ score indicates stronger support for the model. We calculated the distance of each model ($\Delta$AIC$_C$) from the model with the lowest AIC$_C$ value, as well as the Akaike weight ($w_i$), which is the relative likelihood of a given model, given the data (see Burnham and Anderson 2002). We considered models with $\Delta$AIC$_C \leq 2$ as having support from the AIC analysis. For each supported candidate model, we calculated regression coefficients and 95% confidence intervals. Because, multiple models often provide reasonable fits to the data, we combined information from all models using

model averaging to generate parameter estimates, their variances, and 95% confidence intervals. As a measure of variable importance, we used the cumulative Akaike weights for each variable. We considered a variable strongly supported if its model-averaged confidence intervals did not include zero and its cumulative Akaike weight was >0.7, moderately supported if its cumulative Akaike weight was >0.7, and not supported if neither of these criteria were met (Marchetti et al. 2004).

## Results

Total phosphorus concentrations, total nitrogen concentrations, and conductivity were significantly higher in wetlands within the agricultural transect, whereas wetlands in the forested transect tended to be larger in surface area (Table 1). Mean distance to the Mississippi River did not differ between the two transects. We encountered amphibians at 34–40 wetlands and identified 6 species: green frogs (*L. clamitans*), northern leopard frogs (*L. pipiens*), American bullfrogs (*L. catesbeianus*), wood frogs (*L. sylvaticus*), American toads (*A. americanus*), and gray treefrogs (*Hyla versicolor*). Frog abundances averaged $5.56 \pm 1.93$ and $1.67 \pm 0.34$ individuals min$^{-1}$ in the agricultural and forested transects, respectively, while larval amphibian abundances averaged $0.63 \pm 0.49$ and $0.063 \pm 0.02$ individuals sweep$^{-1}$ in the agricultural and forested transects, respectively. We identified 11 gastropod species: *Lymnaea collumela* (2 sites), *Lymnaea humilis* (1 site), *Lymnaea megasoma* (2 sites), *Physa acuta* (2 sites), *Physa gyrina* (16 sites), *Gyraulus parvus* (10 sites), *Helisoma anceps* (23 sites), *Helisoma campanulata* (10 sites), *Helisoma trivolvis* (15 sites), *Planorbula armigera* (1 site), and *Bellamya chinensis* (1 site). Total gastropod abundances averaged $2.83 \pm 1.27$ and $0.65 \pm 0.21$ individuals sweep$^{-1}$ in the agricultural and forested transects, respectively. The average number of gastropod species encountered per site along each transect was 2.08.

We necropsied 160 amphibians and isolated 12,109 larval trematodes representing six taxa: *R. ondatrae*, echinostomes (possible mixture of *Echinostoma* spp. and *Echinoparaphyium* spp.), *Fibricola* sp., *Alaria* sp., *Apharyngostrigea pipientis*, an unidentified plagiorchid and an unidentified strigeid (Fig. 2). We also found adult trematodes (*Haematoloechus* spp.), nematodes (*Oswaldocruzia* spp., an unidentified spiruid, and tadpole pinworm), several protists and leeches, but we focus here on larval
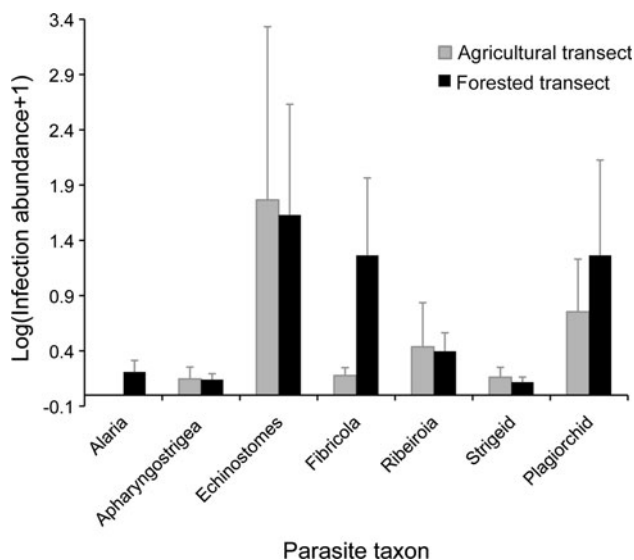
**Figure 2.** Abundance of trematode parasites in amphibians from wetlands along the forested and agricultural transects. Presented are the mean abundances ($\log_{10}$-transformed + 1) and standard errors of each larval trematode group encountered among the 40 wetlands. Data from a total of 160 necropsied amphibians.

trematodes because amphibians acquire them during aquatic stages and they have similar life histories. Of sites that supported frogs (34 of 40 sites), we found *R. ondatrae* at 6 of 18 agricultural sites (33.3%) and at 11 of 16 forested sites (68.8%). At sites supporting *R. ondatrae*, average abundances were 4.90 metacercariae individual$^{-1}$ (SE = 4.2) and 2.18 metacercariae individual$^{-1}$ (SE = 0.58) for agricultural and forested, respectively. We detected echinostome infections in frogs from 17 of 18 agricultural sites and 14 of 16 forested sites, with average infection intensities of 60.8 (SE = 35.7) and 43.3 (SE = 10.3) metacercariae individual$^{-1}$, respectively (Fig. 2).

We collected enough frogs at 32 sites to generate unbiased chao2 estimates of larval trematode species richness. Twenty-two of these sites had sample-based rarefaction curves that clearly reached an asymptote, 3 began to level off but did not reach an asymptote, and 7 had no asymptotic behavior. In most cases (27 of 32), chao2 estimates agreed closely with our original richness estimates, and the average difference between estimated (chao2) and observed richness was 0.61 species. We therefore used the uncorrected richness estimates as this allowed use of the entire data set (although results do not change appreciably if we used the chao2 estimates). This finding was reinforced by the exhaustive data set from Eden Prairie, Minnesota, which showed that five hosts provided a reasonable estimate of species richness (5 hosts mean chao2 ± 1

SD = 4.51 ± 0.44) as compared with larger sample sizes (e.g., 10 hosts yields a richness estimate of 4.77 ± 0.21) (Fig. 3a). Four of the five larval trematodes (*Ribeiroia*, *Echinostoma*, *Fibricola*, and an unidentified plagiorchid) had high infection prevalences (83–99%; $n = 78$ necropsied *L. pipiens*) and were detected even with low sampling effort. One parasite (*Alaria* sp.), however, was present in <10% of examined frogs and required more samples for adequate detection.

Estimates of parasite abundance from five hosts exhibited considerably more variance relative to those for species richness. Among our study sites in Wisconsin, the coefficient of variation (CV) (calculated as the standard deviation divided by the mean) for *Ribeiroia*, *Echinostoma*, and total larval trematode abundance averaged 1.61, 0.75, and 0.51, respectively. Based on the bootstrapping analysis from the intensively studied pond in Eden Prairie, a sample size of five hosts yielded a CV of 0.73 for *Ribeiroia* and 0.57 for *Echinostoma*, while an increase to 40 hosts decreased these values to 0.18 and 0.14, respectively (Fig. 3b). These findings suggest that parasite abundance among individual hosts is highly variable, which should be expected based on the aggregated distribution of parasites within hosts (Poulin 2007), and that the analyses involving abundance are at higher risk of type II (false negative) results.

## Model Selection and Analysis

For response variables related to trematode presence and abundance, models that combined definitive host factors (e.g., land use and spatial position) and intermediate host factors (e.g., amphibian and snail communities) generally had the greatest support (Table 3). Diagnostics such as cumulative Akaike weights and variable confidence intervals consistently identified forested area and/or proximity to the Mississippi River as among the most important variables, with secondary importance on snail and amphibian densities (Fig. 4; Table 4). Because multiple models were often comparably well supported in the analysis, we used a model-averaging approach that incorporated information from all models to estimate parameter coefficients and standard errors (see Table 4) (Burnham and Anderson 2002). This approach is especially helpful in generating robust parameter estimates in cases where multiple models have comparable support, as seen here.

There were three well-supported models describing overall trematode species richness within amphibian hosts that were within 2 $\Delta AIC_C$ of one another (Table 3). Model
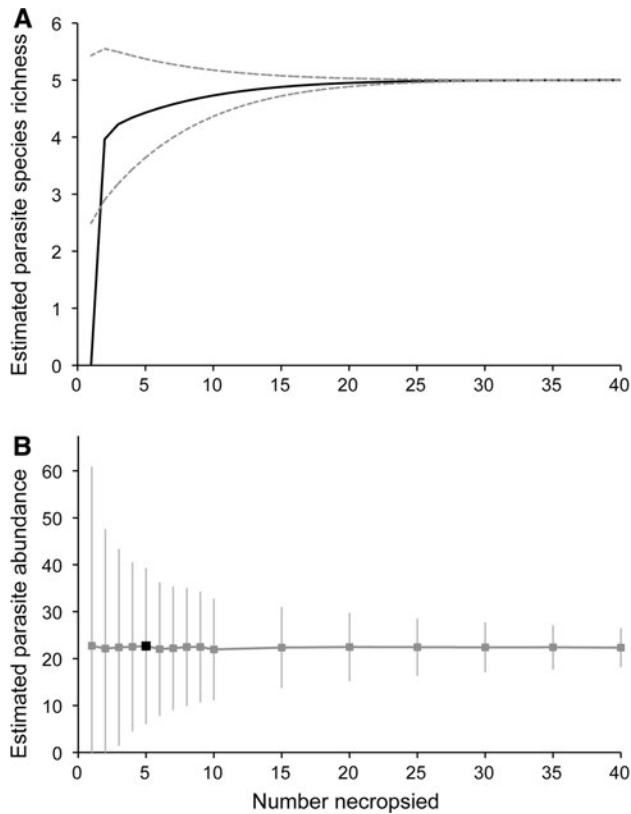
Figure 3. **a** Rarefaction curve generated from an intensively studied wetland in Eden Prairie, Minnesota. Presented is the influence of sample size (i.e., number of necropsied frogs) on estimates of species richness. *Dashed lines* represent lower and upper 95% confidence limits assuming true species richness is given by chao2 estimates, **b** influence of sample size on estimates of parasite abundance. Presented is the estimate of mean *R. ondatrae* abundance (±1 SD) as a function of sample size for 5,000 draws with replacement. The total number of necropsied frogs was 106 leopard frogs (*L. pipiens*).

10 included the amount of forested area and proximity to the Mississippi River, both of which positively affected trematode species richness. Models 19 (forested area, proximity to the Mississippi River, density and richness of snails, and the density of tadpoles) and 24 (forested area, proximity to the Mississippi, and total phosphorus) also had support (Table 3). Based on model averaging, variables hypothesized to affect definitive host activity, including proximity to the Mississippi and forested area, had consistently strong and positive effects on trematode richness with cumulative Akaike weights approaching 1 (0.86 and 0.99, respectively) (Table 4).

For overall trematode abundance (aggregated among species), the best-supported models were 16 and 17, which were within 1 $AIC_C$ unit and included definitive host factors (forested area in model 16 and forested area and

proximity to Mississippi River in model 17) as well as intermediate host factors (snail density and richness, tadpole and frog density) (Table 3). The most supported predictors within these models were forested area (+) and the density of tadpoles (+), with moderate support for snail density (+), frog density (+), and snail richness (−) (Table 4).

Models to predict individual trematode groups yielded similar results. The presence of *R. ondatrae* was best predicted by models 2, 10, and 24, which included positive effects for proximity to the Mississippi River (models 2, 10, and 24), forested area (10, 24), and total phosphorus concentrations (24 only). The abundance of *R. ondatrae* was best predicted by models 15 and 18, both of which included definitive and intermediate host factors: proximity to the Mississippi River (15 and 18), forested area (18 only), the density and richness of snails (15 and 18), the density of frogs (15 and 18) and the density of tadpoles (15 only) (Table 3). Strongly supported variables included proximity to the Mississippi River (+), with moderate support for forested area (+), snail density (−), frog density (−), and snail richness (+) (Table 4). Similarly, the highest-ranking models predicting echinostome abundance came from the definitive and intermediate host suite of models (16 and 17), although forested area was a more important contributing variable to parasite abundance than was proximity to the Mississippi River, while snail, tadpole, and frog density were the most important intermediate host variables (all with positive coefficients) (Tables 3, 4). Although, not included in our original information-theoretic approach, the abundance of *Alaria*, *Fibricola*, and an unidentified plagiorchid were all significantly greater along the forested transect (post hoc *t* tests, all $P < 0.05$) (Fig. 2).

When we repeated the analyses for trematode richness and the abundance of *Ribeiroia*, echinostomes, and all larval trematodes combined using only wetlands that supported enough *L. clamitans* for dissection (i.e., excluding dissections of other amphibian species, $n = 28$ wetlands), results were generally similar although there were some changes in which models were best supported by the data (see Supplementary Materials for full results). For trematode abundance (aggregated among species), models 1 (Global) and 19 (DH + IH) were also within 2 $\Delta AIC_C$ units of 16 and 17. This change placed a greater emphasis on the role of proximity to the Mississippi (cumulative Akaike weight = 0.863) and a lesser emphasis on frog density. Other predictors remained the same. For *Ribeiroia*

**Table 3.** Highest ranking models used to predict each response variable.

| Response | Model | Category | RSS | $k$ | $AIC_C$ | $\Delta AIC_C$ | $w_i$ |
|---|---|---|---|---|---|---|---|
| Trematode richness | 10 | DH | 1.001 | 5 | −218.4313 | 0 | 0.2966 |
| | 24 | DH + PC | 0.965 | 6 | −217.8901 | 0.5911 | 0.2207 |
| | 19 | DH + IH | 0.877 | 8 | −217.4318 | 1.049 | 0.1755 |
| Trematode abundance | 17 | DH + IH | 6.94 | 9 | −71.638 | 0 | 0.5224 |
| | 16 | DH + IH | 7.479 | 8 | −70.7718 | 0.8661 | 0.3388 |
| Ribeiroia presence | 24 | DH + PC | 31.72 | 6 | 46.8311 | 0 | 0.3257 |
| | 10 | DH | 35.263 | 5 | 47.4059 | 0.5747 | 0.2444 |
| | 2 | SV | 39.315 | 4 | 48.6943 | 1.8632 | 0.1283 |
| Ribeiroia abundance | 15 | DH + IH | 1.95 | 8 | −172.6201 | 0 | 0.4069 |
| | 18 | DH + IH | 1.957 | 8 | −172.3765 | 0.2437 | 0.3602 |
| Echinostome abundance | 16 | DH + IH | 7.76 | 8 | −68.4114 | 0 | 0.4217 |
| | 17 | DH + IH | 7.462 | 9 | −66.9966 | 1.4148 | 0.2079 |

Presented here is the ''best'' model for each response (based on $AIC_C$ values) and any models within 2 $AIC_C$ units of the best model. Descriptive diagnostics include the Residual Sum of Squares (RSS, for logistic regression analyses the −2log likelihood is presented), the number of parameters ($k$), the Akaike information criterion adjusted for small sample sizes ($AIC_C$), the difference between a given model's $AIC_C$ value and the lowest $AIC_C$ value ($AIC_C$), and the Akaike weight of the model ($w_i$). For complete descriptions of each model, see Table 2.
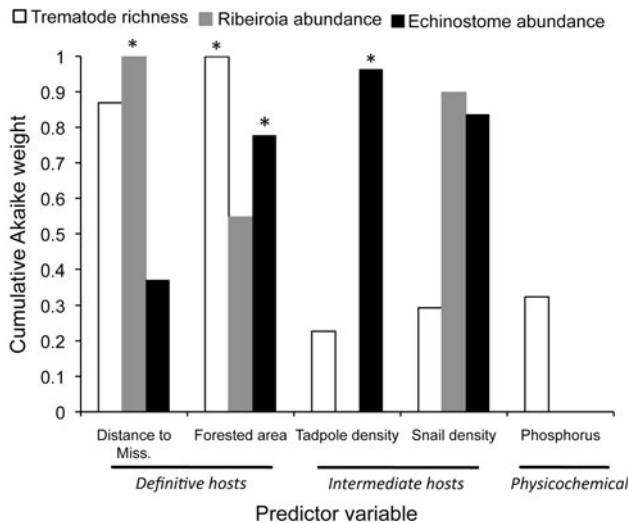


**Figure 4.** Cumulative Akaike weights of independent predictors from the best-supported models for trematode richness, Ribeiroia abundance, and echinostome abundance. Maximum possible value equals 1. * indicate that the 95% confidence interval for the predictor did not include zero, while the labels on the x-axis identify the specific predictor variable and the category of hosts on which it likely acts.

abundance, models 2 (SV) and 10 (DH) were well supported alongside the original results of models 18 and 15. This did not alter the variables considered to be well supported by the data. For echinostome abundance, models 19 (DH + IH) and 15 (DH + IH) replaced models 16 (DH + IH) and 17 (DH + IH) as the most highly ranked

models, which served to add distance to the Mississippi as a supported predictor. For trematode richness, models 10 (DH), 24 (DH + PC), and 19 (DH + IH) were replaced by models 16 (DH + IH), 3 (SV), and 18 (DH + IH), which maintained a heavy weighting on forested area (cumulative Akaike weight = 0.999) but emphasized snail density more strongly than distance to Mississippi (see Supplementary Materials). In general, model-averaged estimates of predictor coefficients were similar between the two sets of analyses although the 95% confidence intervals tended to increase with the reduction in sample size.

## DISCUSSION

By analyzing parasite richness and occurrence data using a competing-hypotheses approach, our results provide evidence that variables affecting both intermediate- and definitive hosts influenced the distribution and abundance of trematode parasites in amphibians. Models that included factors influencing both definitive host activity and intermediate host abundance generally outperformed models involving only single host groups or environmental variables. Both parasite species richness and aggregated abundance were higher in wetlands within the forested transect, likely because forested areas support diverse bird and mammal definitive host communities (Koprivnikar et al. 2006; King et al. 2007, 2010) (although we cannot rule out

**Table 4.** Model-averaged coefficients, standard errors, and 95% confidence intervals for the best-supported independent predictors for each response variable.

| Response variable | Variable | Model-averaged coefficient | Weighted unconditional SE | Lower 95% | Upper 95% | Cumulative Akaike weight |
|---|---|---|---|---|---|---|
| Trematode richness | Distance to Mississippi | −0.160 | 0.1037 | −0.3626 | 0.0419 | 0.869 |
| | **Forested area** | **0.634** | **0.2061** | **0.2322** | **1.0362** | **0.999** |
| Trematode abundance | **Forested area** | **1.632** | **0.5639** | **0.5324** | **2.7318** | **0.963** |
| | Snail density | 0.337 | 0.3830 | −0.4103 | 1.0834 | 0.956 |
| | Frog density | 0.608 | 0.6787 | −0.7153 | 1.9315 | 0.908 |
| | **Tadpole density** | **1.066** | **0.5325** | **0.0282** | **2.1048** | **0.942** |
| | Snail richness | −0.027 | 0.0871 | −0.1971 | 0.1426 | 0.952 |
| *Ribeiroia* presence | **Distance to Mississippi** | **−0.325** | **1.4904** | **−6.1606** | **−0.3479** | **0.859** |
| | Forested area | 6.617 | 3.8423 | −0.8752 | 14.110 | 0.742 |
| *Ribeiroia* abundance | **Distance to Mississippi** | **−0.490** | **0.1567** | **−0.7956** | **−0.1846** | **0.999** |
| | Snail density | −0.284 | 0.1850 | −0.6453 | 0.0765 | 0.898 |
| | Frog density | −0.191 | 0.2437 | −0.6662 | 0.2845 | 0.833 |
| | Snail richness | 0.083 | 0.0441 | −0.0029 | 0.1691 | 0.898 |
| Echinostome abundance | **Forested area** | **1.135** | **0.5669** | **0.0299** | **2.2408** | **0.778** |
| | Snail density | 0.517 | 0.3935 | −0.2497 | 1.2851 | 0.837 |
| | **Tadpole density** | **1.193** | **0.5560** | **0.1082** | **2.2767** | **0.964** |
| | Frog density | 0.482 | 0.5746 | −0.6384 | 1.6027 | 0.789 |
| | Snail richness | −0.047 | 0.0885 | −0.2191 | 0.1260 | 0.797 |

Bolded variables are those with high cumulative Akaike weights and 95% confidence intervals that do not include zero (considered to have strong support). Unbolded variables are those with high cumulative Akaike weights but 95% confidence intervals that include zero (considered to have moderate support).
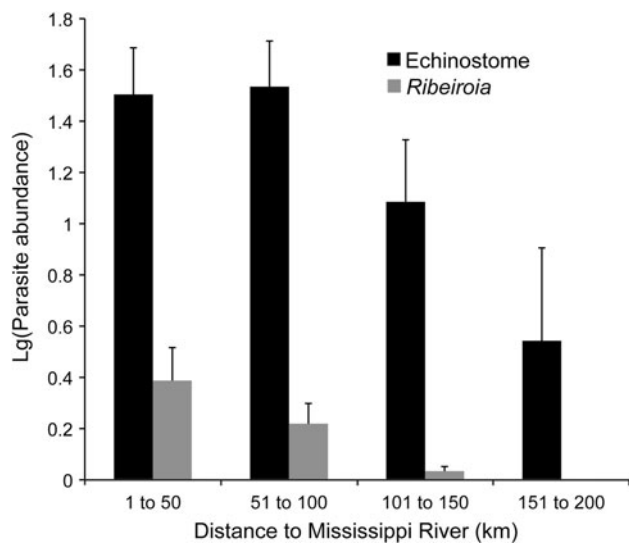
**Figure 5.** Relationship between the distance of a wetland from the Mississippi River (a geographic proxy for the Mississippi Flyway) and the mean abundance ($\log_{10}$-transformed + 1) of Ribeiroia and echinostome infections in necropsied amphibians.

the possibility that additional differences between the two transects contributed to this pattern). Similarly, marine shorebird and gull activity has been reported to relate positively to the trematode infection prevalence in first intermediate host snails (Hoff 1941; Latham and Poulin 2003; Skirnisson et al. 2004; Fredensborg et al. 2006) and second intermediate host sand crabs (Smith 2007). However, the effects of land use on amphibian trematodes were nested within a larger, regional-scale pattern, such that a wetland's proximity to the Mississippi Flyway was a significant positive predictor of both trematode species richness and abundance (Fig. 5). This was true for wetlands in both the agricultural and forested transects, with the steepest decline in infection after 100 km from the Mississippi River. Proximity to migratory bird flyways has also been linked to the occurrence of *Leyogonimus polyoon*, an emerging pathogenic trematode that causes mortality among American coots (Cole 2002; Friend et al. 2001), and to the avian schistosomes responsible for cercarial dermatitis ("swimmer's itch") in humans (Jarcho and van Burkalow 1952; Chu 1958).

At the local wetland scale, larval trematode distributions were related to factors affecting intermediate host abundance, including total phosphorus concentrations and snail and larval amphibian density. Higher densities of intermediate hosts likely increased the transmission of trematode parasites, which move among hosts using a combination of free-living infectious stages (e.g., miracidia

and cercariae) and trophic transmission, in which predatory definitive hosts consume infected intermediate hosts. Higher concentrations of phosphorus, often associated with runoff from agricultural fertilizers or livestock, can enhance snail and tadpole host abundance by increasing algal growth (e.g., Johnson et al. 2007). Similarly, Fredensborg et al. (2006) reported that the spatial distributions of birds influenced trematode prevalence in intertidal communities at large spatial scales, while physical environmental factors contributed to infection patterns at smaller scales. Also working in marine communities, Thieltges and Reise (2007) found evidence that first intermediate hosts were a strong determinant of infection levels in second intermediate hosts among sites, with small-scale differences in abiotic properties further contributing to observed infection levels.

The parasites included in our analyses differed significantly in their responses to specific predictor variables, likely as a function of their respective life cycles. For example, *R. ondatrae* presence was positively related to proximity to the Mississippi Flyway, the amount of forested area, and phosphorus concentrations. Trematode community diversity also followed these patterns, with forested area and distance to the Mississippi Flyway as strong predictors in most models, highlighting the dependency of many trematode life cycles on bird definitive hosts. Counter to our hypotheses and the results for echinostomes, *R. ondatrae* infection abundance was negatively influenced by snail and amphibian intermediate host abundance, although these variables had only moderate support in the analysis with confidence intervals that included zero. For echinostomes, our analyses revealed a stronger association with the amount of forested area and intermediate host abundance, rather than with distance to the Mississippi Flyway. This could owe to the low host specificity of echinostomes, which often use mammals and birds as definitive hosts (Kanev et al. 1995; Johnson and McKenzie 2008) and might therefore be less influenced by bird migratory pathways.

As with any study involving a subsampling approach, it is important to acknowledge uncertainties in the data that could influence the results. To evaluate our approach, we used an exhaustive data set from an intensively studied wetland located near the two transects. These data, in conjunction with rarefaction diagnostics used for the broad-scale survey data, indicate that our estimates of species richness were robust, despite the small number of hosts sampled per site. This likely reflects the fact that, for

metamorphosing frogs sampled in small wetlands, larval trematode infection prevalence increases rapidly with parasite abundance, such that rare species are less likely to skew the results (although note that this is less likely to be true for parasites acquired through diet or after metamorphosis). However, the data for parasite abundance were much more variable, with standard deviations often only slightly smaller than the mean and variance measures that decreased slowly with additional increases in sample size. This is not surprising given the tendency of parasite infections to be highly aggregated within hosts, often following the negative binomial distribution (e.g., Poulin 2007), but suggests that results stemming from theses analyses need to be interpreted with caution, especially for weakly supported effects or those not found significant (i.e., the risk of type II error is increased). However, the risk of false positive (type I error) results should not increase given the lack of any systematic biases, particularly given the incorporation of a model-averaging approach (Burnham and Anderson 2002). These observations, in combination with the similarity of the results for parasite richness and abundance, suggest that strongly supported variables such as land use and flyway proximity play important roles in driving patterns of infection within this study.

Our results have additional relevance for understanding how ongoing land use transformations influence the distribution of amphibian pathogens. Previous study has found that agricultural activity, including runoff of nutrients and pesticides, can cause increased trematode infection in amphibians (Kiesecker 2002; Johnson et al. 2007; Rohr et al. 2008). Indeed, the density of snails and amphibians in our study tended to be greater on agricultural wetlands than on forested wetlands (Table 1). A priori, we might therefore expect to find increased infections in wetlands from the agricultural transect. However, many of the trematodes in this study were more likely to occur and more abundant on forested wetlands. This finding, in combination with the overriding importance of forested area and proximity to the Mississippi Flyway in our models, suggests that even while agricultural land-use can enhance the abundance of intermediate hosts, factors affecting definitive host activity strongly influence the input of parasites to a wetland, consistent with previous studies on trematode communities (Hechinger and Lafferty 2005; Fredensborg et al. 2006; King et al. 2010). We cannot rule out, however, that such differences might also result from unknown factors associated with pesticides, which we did not measure, from the extremely high nutrient concentrations

observed in the agricultural ponds, which were higher than previous studies exploring the nutrient–parasite relationship ($>300 \, \mu g \, l^{-1}$), or from additional, unmeasured differences between the two transects, which were necessarily separated geographically (Pietrock and Marcogliese 2003; Christin et al. 2003; Gendron et al. 2003; Linzey et al. 2003; King et al. 2007, 2010).

Intriguingly, however, while agricultural and forested wetlands had relatively similar average infection levels for *R. ondatrae* and the echinostomes, sites along the agricultural transect had higher variance in parasite abundance (Fig. 2). The coefficient of variation for the abundance of both parasites was ∼3× greater among agricultural wetlands than forested wetlands. Moreover, the maximum observed infections for *R. ondatrae* (56 metacercariae in an individual frog with a wetland mean of 26 metacercariae frog$^{-1}$) and for echinostome infections (795 metacercariae in an individual frog with a wetland mean of 616 metacercariae frog$^{-1}$) occurred on the agricultural transect. The wetland with the highest recorded *R. ondatrae* infection was also the only site at which obvious limb malformations were observed. This may indicate that, when definitive host activity is sufficient, agricultural areas create the potential for high transmission because of their favorable effects on intermediate hosts. While more replication is needed to critically evaluate this pattern, these results suggest that a perspective focused only on mean infection values might differ from one attempting to predict the occurrence of "hotspot" sites, where parasite-induced pathology is most likely to occur. Intensive forms of human land use, such as agricultural development, may function to enhance variation in parasite infection, leading to more "extreme" outcomes such as parasite absence in some cases or infection epizootics in others.

## SUMMARY

Our results indicate that predictors operating across multiple spatial extents interact to create spatial heterogeneity in amphibian infections. At the local scale, eutrophication caused by nutrient addition from agricultural land-use provided a favorable environment for intermediate host snails and larval amphibians (Johnson and Chase 2004; Johnson et al. 2007; Rohr et al. 2008). At the watershed scale, land use patterns and the amount of agricultural versus forested area influenced the abundance of definitive hosts, which determine the abundance of many parasites by

contributing stages infective to intermediate hosts (Smith 2007). Finally, the regional patterns of parasite occurrence and abundance were sharply affected by proximity to the major bird flyway, especially for parasite diversity and the abundance of bird-dependent parasites. Given the importance of multi-host parasites to wildlife and human health, as well as the ongoing changes in land use worldwide (Holmes 1996), we emphasize the importance of continued research on landscape epidemiology to identify important drivers of infection and how they vary in response to a changing environment.

## ACKNOWLEDGMENTS

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