Effects of wetland vs. landscape variables on parasite communities of *Rana pipiens*: links to anthropogenic factors

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Abstract. The emergence of several diseases affecting amphibian populations worldwide has prompted investigations into determinants of the occurrence and abundance of parasites in frogs. To understand the spatial scales and identify specific environmental factors that determine risks of parasitism in frogs, helminth communities in metamorphic frogs of the northern leopard frog (*Rana pipiens*) were examined in relation to wetland and landscape factors at local (1 km) and regional (10 km) spatial extents in an agricultural region of Minnesota (USA) using regression analyses, ordination, and variance partitioning techniques. Greater amounts of forested and woody wetland habitats, shorter distances between woody wetlands, and smaller-sized open water patches in surrounding landscapes were the most consistently positive correlates with the abundances, richness, and diversity of helminths found in the frogs. Wetland and local landscape variables were suggested as most important for larval trematode abundances, whereas local and regional landscape variables appeared most important for adult helminths. As previously reported, the sum concentration of atrazine and its metabolite desethylatrazine, was the strongest predictor of larval trematode communities. In this report, we highlight the additional influences of landscape factors. In particular, our data suggest that anthropogenic activities that have resulted in the loss of the availability and connectivity of suitable habitats in the surrounding landscapes of wetlands are associated with declines in helminth richness and abundance, but that alteration of wetland water quality through eutrophication or pesticide contamination may facilitate the transmission of certain parasite taxa when they are present at wetlands. Although additional research is needed to quantify the negative effects of parasitism on frog populations, efforts to reduce inputs of agrochemicals into wetlands to limit larval trematode infections may be warranted, given the current high rates of amphibian declines and extinction events.

Key words: amphibian; CANOCO; echinostomatid; FRAGSTATS; landscape analysis; Minnesota, USA; parasite community structure; Rana (=Lithobates) pipiens; Ribeiroia ondatrae; trematode.

Introduction

How land use affects the distributions of infectious diseases is an increasingly important issue in ecology and conservation biology (Hess et al. 2002, Ostfeld et al. 2005, Collinge 2009). Loss and fragmentation of natural habitats may result in changes in the abundances and interactions between hosts, vectors, and parasites within remaining habitat patches, leading to changes in the presence and severity of diseases. For instance, increases

in the Lyme disease spirochete (Allan et al. 2003), raccoon roundworms (*Baylisascaris procyonis*; Page et al. 2001), and primate gastrointestinal helminths (Gillespie and Chapman 2006) have been detected in patches of forest that remain after land has been cleared for agriculture because the rates of contact between hosts and parasites are altered in the remaining forested patches. Disease transmission and spread across landscapes may also be modified by changes in the connectivity and subsequent host and parasite movements among remaining habitat patches (Taylor and Merriam 1996, Langlois et al. 2001, Collinge et al. 2005, Ostfeld et al. 2005, Collinge 2009). Therefore, the influence of land use change on disease dynamics should relate to changes in the quality of habitat patches as well as the surrounding landscape. A complete understanding of the interactions between local patch characteristics and landscape context on disease dynamics, however, is lacking (Ostfeld et al. 2005). Moreover, the

spatial scales that influence particular life stages of parasites and host–parasite interactions in general are poorly understood, such that the extent to which landscape modifications affect infections and disease are not known.

Amphibians are a class of vertebrates for which the negative effects of loss and fragmentation of natural habitats have been well documented (Collins and Storfer 2003, Stuart et al. 2004). For many amphibian species, aquatic systems serve as critical habitats for breeding, larval development, and overwintering, whereas terrestrial systems may be necessary for adult foraging and hibernation. Amphibians also are hosts to a wide variety of helminth parasites, with varying transmission strategies, and for which transmission may occur in the aquatic or terrestrial environments (McKenzie 2007). Thus, it is likely that the transmission dynamics of amphibian parasites are influenced by both wetland (e.g., patch) and surrounding landscape factors.

Previous investigations have indicated that the alteration of wetland water quality has contributed to the increased transmission of helminth parasites in amphibians, suggesting that patch conditions are particularly important. For instance, eutrophication associated with agricultural run-off may result in higher infection prevalence and intensities of the trematode, *Ribeiroia ondatrae*, in snail and tadpole populations, contributing to the emergence of limb malformations in amphibians (Johnson et al. 2002, 2007). Similarly, in a previous report, we identified the herbicide, atrazine, as a contaminant in wetlands that may increase the exposure and susceptibility of tadpoles to larval trematode infections (Rohr et al. 2008b). In contrast, King et al. (2007) and King et al. (2010) found that increased agriculture in the surrounding landscapes around wetlands was highly important because it generally resulted in a decline in helminth parasites of amphibians, presumably because of a loss of natural habitats for the definitive hosts of many of the parasites found in amphibians. McKenzie (2007) reported that amphibian parasite responses to agriculture (specifically, cattle grazing) varied depending on the life cycles of the parasites, but that the majority of parasite species were more abundant and parasite communities were more species rich in frogs collected at cattle grazed pasture sites compared to forested sites. Therefore, these previous studies suggest that both wetland conditions and landscape context may influence disease dynamics in amphibians; however, we know little about the relative contributions of patch vs. landscape factors to disease dynamics in amphibians.

The current study aimed to improve the understanding of the interplay between wetland and landscape factors on amphibian parasite communities. We investigated the importance of landscape context at two spatial extents (1 and 10 km) relative to wetland (patch) characteristics in a region fragmented to varying degrees by agriculture. We conducted regression analyses and

used a model-selection approach to identify the environmental variables that best explained measures of parasite community structure at each spatial scale. We then used a variance partitioning technique to determine the relative contributions of the different models to the variation in parasite community structure. The goals of our study were to (1) identify specific wetland and landscape features that were markedly associated with parasitism in amphibians, and (2) determine the spatial scale(s) that appeared to be most informative with regard to amphibian parasite communities. This approach differs from previous investigations that have simply attempted to identify associations between agricultural land use and amphibian parasite populations or communities (Koprivnikar et al. 2006, King et al. 2007, 2010, McKenzie 2007, Rohr et al. 2008b), although based on these previous studies, we predicted that decreasing parasitism would be associated with a loss of natural habitats in the surrounding landscapes and increasing parasitism would be associated with nutrient enrichment and pesticide exposure within the wetland. Determining the specific spatial scales of host–parasite responses to habitat changes will help plan effective management and disease control and prevention strategies.

Materials and Methods

Study system

The current investigation was conducted with data obtained during an amphibian health assessment study completed in 1999 (Beasley et al. 2001). Metamorphic frogs (post-Gosner [1960] stage 44) were selected for study because these individuals would have spent their larval periods, and therefore, acquired parasites at a particular wetland, allowing for the analysis of parasite community attributes in relation to environmental conditions associated with specific wetlands. This would be in contrast to adult frogs that may have visited and acquired parasites in many different wetlands and terrestrial habitats that varied in environmental quality. In addition, the removal of small numbers of metamorphic frogs from most populations is expected to have negligible effects on existing populations because of their naturally high attrition rates. *Rana* (=*Lithobates*, Frost 2009) *pipiens* was chosen for study because the species is widely distributed in the Midwest and it inhabits a diversity of habitat types, including those disturbed by agriculture. Furthermore, the status of this species is of concern in parts of its range in the Midwest (Seburn 1992, Mossman et al. 1998).

Study area

Wetlands located within the Eastern Broadleaf Forest Province of Minnesota, USA were studied (Appendix A). This region of Minnesota is a largely agricultural area, with most crop acreage used for maize and soybean production (U.S. Department of Agriculture 2002). In this part of the state, much of the landscape

has been converted from one once dominated by forest and prairie to one dominated by row crops and pastureland, leaving natural areas highly fragmented. The majority of wetlands in the southern part of the state, which included our study area, have also been drained (Dahl and Allord 1997). Our study area also included the highly urbanized area of Minneapolis–Saint Paul, and surrounding areas that experienced suburban population growth during 1990–1998 (Hibbs 2000). Though we were not able to conduct a before-and-after comparison of frog parasite communities to directly link parasite responses to such land use changes, we were able to examine relationships between parasites and characteristics of different surrounding landscapes to make inferences regarding how parasite populations might respond to loss and fragmentation of natural habitats.

The methods used to select wetlands for study have been described previously (Rohr et al. 2008b). Briefly, five wetland clusters consisting of 6–8 wetlands each were established in areas, loosely defined by fourth-order watershed classifications. Within each cluster, we attempted to select wetlands that represented a gradient of low (<20%) to high (>35%) percentage composition of surrounding agriculture. To reduce the potential effects of spatial autocorrelation, sites chosen for study were also at least 2 km apart from each other. Two km was deemed appropriate to reduce spatial autocorrelation because this exceeds the known dispersal abilities of leopard frogs (Merrell 1977).

Parasite data

Parasite community data were obtained from recently metamorphosed leopard frogs collected from 18 wetlands over a 4-week period in July–August 1999. These were wetlands where five or more frogs were necropsied and examined for parasites, though for the majority of our wetlands ($n = 14$) we examined 14–16 frogs. Frogs were transported in chilled containers from Minnesota to the USGS National Wildlife Health Center (NWHC) in Madison, Wisconsin, USA within two days of capture. Upon arrival, frogs were placed in a refrigerator set at 10°C and necropsied within 5.7 ± 2.43 d (mean ± SD) of collection. Euthanasia was performed by immersing frogs in 1:1000 tricaine methanesulfonate (MS-222). Frogs were weighed to the nearest 0.01 g, measured (snout–vent and total lengths [mm]), and examined for external abnormalities prior to necropsy. Procedures for capture, transport, examination, and euthanasia of the animals were approved by the University of Illinois and NWHC Institutional Animal Care and Use Committees.

Parasitologic exams followed standard protocols. The buccal and abdominal cavities were examined, and all visceral organs, skin, and eyes were removed and inspected for parasites. The remaining frog carcasses were fixed in 10% neutral buffered formalin and cleared and stained using a modified method of Hanken and Wassersug (1981). Encysted metacercariae in the musculature were identified and enumerated by examining the cleared and stained specimens under a dissecting microscope. Differentiation of the metacercariae observed in the cleared and stained specimens was possible because distinguishing morphological characters, such as the excretory vesicle and collar spines were preserved (A. M. Schotthoefer and A. V. Koehler, *personal observations*). Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology at the University of Nebraska–Lincoln (accession number P-2010-021) and cleared and stained frogs have been deposited in the Bell Museum of Natural History, University of Minnesota, Minneapolis–Saint Paul, Minnesota (collection numbers 14624–15168).

Wetland and landscape characteristics

Each wetland was characterized in terms of water quality, sediment composition, pesticide contamination, and biological diversity during three visits in 1999 (March–April, May–June, and July–August). Details concerning the data collected and the protocols used can be found in Rohr et al. (2008b).

Landscape structure, including composition, patch density, and connectivity patterns, was quantified around each wetland using FRAGSTATS (version 2; McGarigal et al. 2002). Land cover data for this purpose were derived from the 1990s National Land Cover Database (NLCD), which was based primarily on 1992 Landsat-5 Thematic Mapper data, with a 30-m resolution (Vogelmann et al. 2001). These data were used to summarize landscapes at 1- and 10-km spatial extents, which we termed local and regional scales, respectively. One-km extents were chosen because these distances are related to the dispersal capabilities of leopard frogs (Merrell 1977), and allowed for an exploration of the effects that adjacent land use had on the parasite communities, such as by potentially altering the water quality within wetlands. Landscapes at 10 km were examined to explore potential impacts of more highly mobile definitive hosts of many frog parasites (e.g., birds and mammals) on the parasite communities. We reclassified the NLCD data into 10 land use classes: agriculture (AG), low-intensity residential (RLD), high-intensity urban (HURB), grassland (GRAS), forest (FOR), shrubland (SHRUB), palustrine-emergent wetland (PEW), woody wetland (WW), open water (OW), and miscellaneous bare rock and quarry surfaces (MISC) (Appendix B) prior to FRAGSTATS analyses.

Data analyses

A large matrix of environmental variables (>240) was available for analysis, comprising three data sets: (1) wetland-specific characteristics, which included water chemistry and soil properties data, as well as taxon-based metrics developed from surveys of wetland biota (e.g., breeding amphibians, macro-invertebrates, and

TABLE 1. Spatial, wetland, local, and regional variables quantified for the 18 surveyed wetlands within the Eastern Broadleaf Forest Province, Minnesota, USA, included in analyses.

Variable code	Definition
Wetland predictors	
FROGS	no. frog and toad species
FWGS	no. freshwater gastropod species
NPLAN	abundance of *Planorbella* snails
VEGS	no. plant species
%TYPH	line transects composed of *Typha* spp. (%)
%PHAL	line transects composed of *Phalaris* spp. (%)
SOILOM	organic matter of soil samples (%)
SUMATRAZ†	sum of atrazine and desethylatrazine (ppb)
SUMPAH†	sum of naphthalene and phenanthrene (ppb)
MAXN	maximum nitrate detected in water (ppm)
MAXP	maximum phosphate detected in water (ppm)
Local predictors‡	
DIST(habitat§)	distance to nearest patch of habitat type
FORLD-1k	land composed of forest (%)
FORPD-1k	patch density of forest
WWLPI-1k	land composed of largest patch of woody wetland (%)
WWMNN-1k	mean distances between patches of woody wetland
OWMPS-1k	mean patch size of open water
PWLD-1k	land composed of palustrine-emergent wetland (%)
AGLD-1k	land composed of agriculture (%)
Regional predictors¶	
FORLD-10k	land composed of forest (%)
WWPD-10k	patch density of woody wetland
WWLPI-10k	land composed of largest patch of woody wetland (%)
WWMNN-10k	mean distances between patches of woody wetland
OWMPS-10k	mean patch size of open water
PWLD-10k	land composed of palustrine-emergent wetland (%)
AGLD-10k	land composed of agriculture (%)
AGPD-10k	patch density of agriculture
AGLPI-10k	land composed of largest patch of agriculture (%)

† Tested for >20 common contaminants, but only these were detected commonly.
‡ Quantified within a 1 km radius around each wetland site.
§ Agriculture (AG), forest (FOR), low-density residential area (RLD), open water (OW), woody wetland (WW).
¶ Quantified within a 10 km radius around each wetland site.

plant communities), (2) local landscape composition and structure variables summarized at 1-km extents, which also included variables that related the positions of wetlands to specific surrounding landscape features, such as distances to nearest forest or wetland patches, and (3) regional landscape variables summarized at 10-km extents.

To reduce the number of environmental variables examined, correlations of variables were screened using Pearson's correlation coefficients and by viewing scatter diagrams constructed to display relationships between variables. When two variables were highly correlated (e.g., $r \geq 0.8$), the variable with higher correlations to our dependent variables was retained for the analyses. Quality of wetland-specific measurements and ease of interpretability of the numerous landscape metrics obtained from the FRAGSTATS analysis were also considered in retaining variables for further evaluation. In addition, we chose to retain wetland variables that were expected to be important based on basic knowledge of parasite life cycles and predictions made in the amphibian health literature (e.g., that anthropogenic chemicals and nutrients should influence parasitism, e.g.,

Kiesecker 2002, Beasley et al. 2005, Johnson et al. 2007). We also retained specific landscape variables to explore not only the potential influence of landscape composition (percent cover of land use types, LD), but also the influence of landscape structure, measured as patch density (PD), largest patch index (LPI), mean patch size (MPS), and mean nearest neighbor patch distances (MNN) for particular land use types; specifically, AG, FOR, WW, PEW, and OW. By examining measurements of composition and structure related to these specific land use types, we hoped to identify the dominant landscape features that most influenced interactions among parasites and their hosts. The final matrices used in our analyses of parasite–environment relationships contained 11 wetland-specific variables, 12 local landscape variables, and 9 regional landscape variables (Table 1).

Wetlands were regarded as experimental units in our analyses. We considered parasite mean abundances, richness, and diversity found in the samples of frogs from wetlands as dependent variables in our analyses. Mean abundance is the mean number of parasite individuals found in all frogs examined (infected and

noninfected frogs), and integrates information about commonality (e.g., prevalence) and severity (e.g., intensity) of infections in host populations (Bush et al. 1997). Richness is simply the number of parasite taxa recovered from frogs at a wetland, and diversity was measured with Shannon's diversity index (McCune and Grace 2002).

To identify relationships among environmental variables and the communities of parasites in leopard frogs, we first conducted principal component analyses (PCAs) followed by redundancy analyses (RDAs) on parasite taxon-specific mean abundance data. PCAs and RDAs are ordination techniques for linear responses to gradients, where the ordination axes are selected sequentially to explain the greatest amount of remaining variation (i.e., axes are canonical). The average response gradient for our parasite mean abundances was <1.73 standard deviations, suggesting the treatment of responses as linear was appropriate. Rare parasite taxa were excluded from these analyses because they can be overly influential in linear ordination techniques. Normal probability plots for the retained parasite taxa revealed no severe deviations from normality or the presence of extreme outliers.

Because there were many more environmental variables than samples (32 vs. 18, respectively), we constrained the RDAs to only those environmental variables that were identified as significant ($P < 0.05$) using Monte Carlo permutations with a forward stepwise selection procedure (999 iterations). Prior to the RDAs, we relativized the mean abundance data by dividing each site's mean abundance by the total abundances across sites. This was done to give each site equal weight in the analysis. Angular transformations were then performed on the resulting proportions. Ordination analyses and hypothesis testing were conducted using CANOCO 4.5 (ter Braak and Smilauer 2002). To display ordination results, we created biplots using CanoDraw 4.12 with a focus on interspecies correlations (ter Braak and Smilauer 2002). The phylotype scores were post-transformed so that correlations of the taxa and environmental variables with the ordination axes could be inferred by perpendicular projection.

If both taxa and environmental similarity covary in geographic space, identifying a causal link between environmental variables and taxonomic composition could become difficult because the compositional similarity might be due to either the environmental similarity or the high probability that sites in close proximity will receive each others' dispersers. Consequently, we evaluated the spatial autocorrelation among mean abundance for each parasite taxon and distances between wetlands using Mantel tests (McCune and Grace 2002) prior to these analyses.

Our preliminary PCAs indicated a separation among taxa that roughly mirrored taxa that use frogs as intermediate hosts and mature in other vertebrate hosts (e.g., larvae of digenetic trematodes, LARVAL) and those that mature in frogs (e.g., adults of digenetic trematodes and nematodes, ADULT) (Fig. 1). Therefore, for subsequent analyses, we focused on the richness and overall mean abundances of these two types of parasites (e.g., LARVAL and ADULT) rather than on individual taxa. However, because of their potential importance to amphibian populations (e.g., Johnson et al. 2002, Skelly et al. 2006, Szuroczki and Richardson 2009), we did examine specific mean abundance–environment relationships for *R. ondatrae* and kidney echinostomatids. We used an informatic theoretic approach to identify models and explanatory variables related to our univariate response variables. We examined only linear models that contained three or fewer explanatory variables within each of the three sets of environmental variables, and identified the most parsimonious model (termed best, here) for each set based on minimum Akaike's Information Criterion, corrected for small-sample size (AIC$_c$; Burnham and Anderson 2002). An overall best model was also selected among models containing three or fewer of the environmental variables in the complete list considered in Table 1. Only models with ΔAIC ≤ 4.01 were retained for further consideration. To evaluate the relative importance of different explanatory variables in the retained models, a model averaging technique was used to calculate model weights (w) and the probabilities that variables should be included in models (w_+) (Burnham and Anderson 2002). Estimated coefficients for each variable were calculated from this reduced set of models using a weighted average of the coefficient values from the models that contained the variable (Burnham and Anderson 2002). These analyses were conducted using the genmod procedure in SAS 9.0 (SAS Institute, Cary, North Carolina, USA).

We used standard multivariate variance partitioning techniques (Borcard et al. 1992) to estimate the variance in parasite abundance, richness, and diversity accounted for by each of the three environmental variable sets alone, as well as confounded among the variable sets. We used the best models identified with the AIC$_c$ model selection methods discussed previously (e.g., wetland, local, and regional best models), then used the program VarPartV1 (*available online*)[8] and the guidance of (Peres-Neto et al. 2006) to partition the variation in the parasite data into eight components: (1) explained solely by wetland variables, (2) explained solely by local variables, (3) explained solely by regional variables, (4–6) explained by sharing of possible pairwise combinations of variable sets, (7) explained by the three-way combination of variable sets, and (8) unexplained variance. Because there is often a positive correlation between the number of explanatory variables in a data set and the amount of variation for which that data set explains, VarPartV1 and Peres-Neto et al. (2006) advance earlier variance partitioning approaches by

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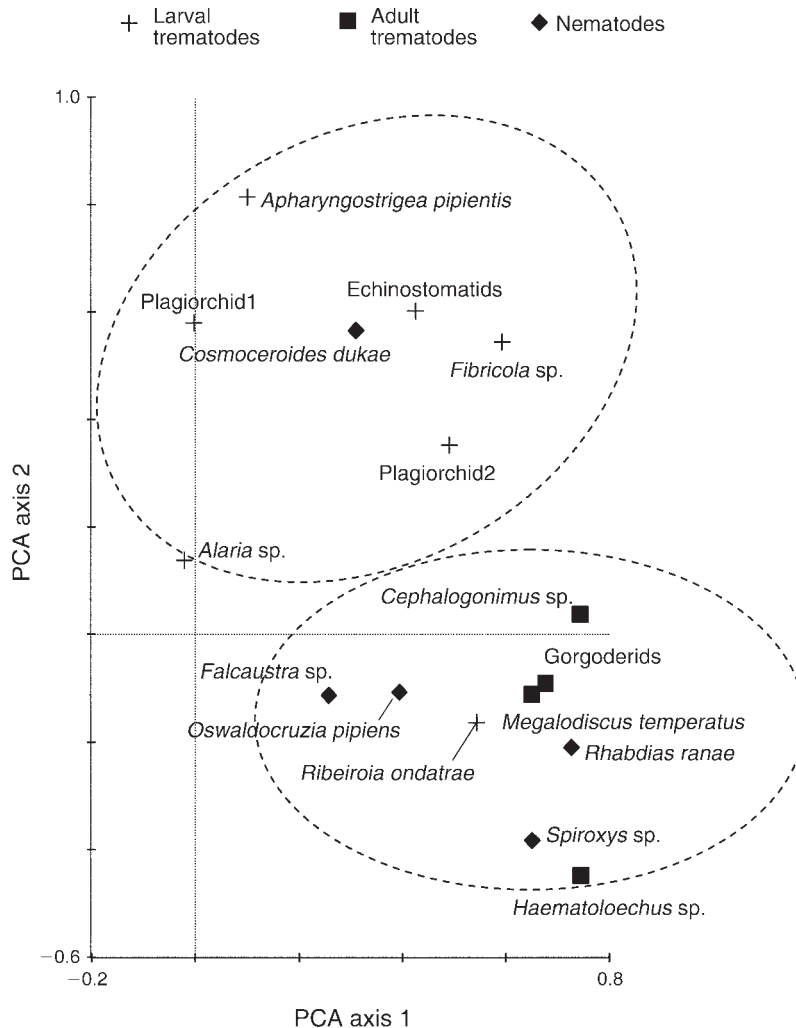[8] ⟨http://esapubs.org/archive/ecol/E087/158/suppl-l.htm⟩

FIG. 1. Principal components analysis results for the leopard frog (*Rana pipiens*) parasite communities based on angular-transformed, relativized species mean abundances per wetland in the Eastern Broadleaf Forest Province, Minnesota, USA. Circles are drawn to highlight the separation of LARVAL vs. ADULT parasite taxa.

adjusting for the number of explanatory variables in each data set using multivariate, adjusted R^2 values. Again, because wetlands in close proximity may have similar parasite communities because of similar environmental characteristics or because they receive parasites from the same dispersing pool, we used Moran I tests to check for spatial autocorrelation among the standardized residuals of our best models prior to the variance partitioning analysis. The absence of spatial autocorrelation would indicate that it was not necessary to control for geographic locations of wetlands, or space sensu Borcard et al. (1992), in these analyses.

RESULTS

General description of parasite communities and landscape structure

Nineteen metazoan parasite taxa were recovered from the leopard frogs considered in this analysis ($n = 237$

frogs; stages 44–46 [Gosner 1960]). Nine of these parasite taxa were classified as larval trematodes (LTREM), four were adult trematodes (ATREM), five were nematodes (NEM), and one was a larval cestode (Table 2). Nearly all of the parasite individuals (97.4%) recovered from frogs were LTREMs.

Ribeiroia ondatrae was found in our study region, although at a low frequency. It was present at a total of five sites included in the analyses. Prevalences (e.g., percentages of frogs infected) at these sites ranged between 6.2% and 100% and mean abundances ranged between 0.06 and 35.2 metacercariae per frog. The most frequently encountered parasites were trematode larvae collectively referred to as echinostomatids, which occur within the kidneys of frogs and may belong to the genera *Echinostoma* and *Echinoparyphium*. The highest overall mean abundances were found for the larvae of *Fibricola* sp. (Table 2). Three parasites were considered rare and

TABLE 2. Taxa, life cycle information, and summaries of occurrence for helminths recovered from leopard frogs (*Rana pipiens*) in analyses of 18 wetland study sites.

Taxon	Parasite class†	Intermediate hosts‡	Definitive hosts§	Prevalence (%) Mean	Prevalence (%) SD	Abundance Mean	Abundance SD	Wetlands with detections (%)
Echinostomatids¶	LTREM	PL, PH, T	B, M	89.4	12.6	89.4	84.5	100
Ribeiroia ondatrae	LTREM	PL, T, F	B, M	10.0	24.8	3.4	9.1	28
Diplostome	LTREM	?	B, M	0.8	3.4	<0.1	0.1	6
Apharyngostrigea pipientis	LTREM	PL, T	B	9.9	14.3	0.8	2.0	39
Clinostomum sp.	LTREM	PL, T	B	2.2	5.6	<0.1	0.1	17
Alaria sp.	LTREM	PL, PH, T	M	21.5	30.7	53.0	184.0	56
Fibricola sp.	LTREM	PH, T	M	50.4	37.3	110.7	170.9	89
Plagiorchid1	LTREM	?	?	48.0	30.6	11.0	17.4	89
Plagiorchid2	LTREM	?	?	55.6	33.2	17.3	35.0	94
Haematoloechus spp.	ATREM	PL, PH, O	F	15.1	16.2	1.9	4.6	72
Megalodiscus temperatus	ATREM	PL, A, T	F	17.4	23.5	0.5	0.8	56
Cephalogonimus sp.	ATREM	PL, T	F	7.6	8.4	0.7	2.0	56
Gorgoderids#	ATREM	CL, T	F	8.9	16.6	1.6	2.9	39
Oswaldocruzia sp.	NEM	NR	F	15.9	15.5	0.7	1.0	78
Falcaustra sp.	NEM	NR	F	7.4	11.7	0.3	0.5	33
Rhabdias ranae	NEM	NR	F	8.3	12.7	0.2	0.3	44
Cosmocercoides dukae	NEM	F, S	F, S	16.8	17.0	0.5	0.9	72
Spiroxys sp. (larval)	NEM	T	TU	17.3	16.1	0.6	1.1	83
Plerocercoid (larval)	CES	CO, T, F	?	5.5	16.0	0.5	2.0	22

† Key: LTREM, larval trematode; ATREM, adult trematode; NEM, nematode; CES, cestode; in analyses, LARVAL, LTREM taxa; ADULT, ATREM and NEM taxa, with exceptions stated in *Results: General description of parasite communities and landscape structure.*

‡ Key: PL, snails belonging to Planorbidae; PH, snails belonging to Physidae; A, Ancylidae mollusks; CL, clams belonging to Sphaeriidae; S, terrestrial snails; T, tadpoles; F, frogs; O, odonate larvae; CO, copepods; NR, not required; ?, hosts unknown. ADULT refers to both adult trematodes and adult nematodes. LTREM is equivalent to LARVAL in the text, and both designations include only larval trematodes.

§ Key: B, bird; M, mammal; F, frog; S, terrestrial snails; TU, turtle.

¶ Includes species belonging to the genera *Echinostoma* and *Echinoparyphium*.

Includes species belonging to the genera *Gorgodera* and *Gorgoderina*.

were excluded from all analyses: *Clinostomum* sp., a diplostome larval trematode, and a plerocercoid cestode; these parasites occurred at 1–3 sites at low prevalences and abundances (Table 2). ADULT taxa included all taxa classified as ATREM and NEM, except *Cosmocercoides dukae*, a nematode that is considered to be an incidental parasite of frogs (Anderson 2000) and *Spiroxys* sp., a larval nematode (Table 2). Species accumulation curves suggested that our frog sample sizes were appropriate for detecting parasite taxa at each wetland, and no correlations were found between frog sample size and parasite abundances, richness, or diversity (Spearman rank tests, all $P \geq 0.15$).

Summaries of the wetland and landscape features considered in the current analysis can be found in Appendix C. In the region of Minnesota studied, the amounts of FOR and WW habitats available were inversely related to the percentage composition of AG land (at 10-km extent, Spearman's rho = −0.89, −0.67; $P < 0.0001$ and 0.003, respectively). Landscapes composed of predominately AG (e.g., >40% AGLD), had patches of AG that were large and contiguous and tended to have fewer patches of FOR and other native habitats, like WW and PEW. Where AG land was less dominant, it existed as numerous, small patches, highly interspersed with other land use types, such that mean nearest neighbor (MNN) distances between WW and PEW patches were shorter. OW patches tended to increase in size with percentage composition of AG, but

decreased in size in the most cultivated landscapes. OW patches also tended to be more distantly separated from other water bodies in landscapes containing more AG.

Determinants of parasite community structure

In the PCA analysis, the first two axes accounted for ~43% of the total variation in the parasite mean abundance data (Fig. 1). The RDA based on parasite mean abundance data for all helminth species accounted for 44% of the variance in the community data (Fig. 2). The variables that were identified as significant predictors (e.g., Ps ≤ 0.05) of parasite community structure in the RDA included: (1) the wetland variables of sum concentration of atrazine and desethylatrazine (SUMATRAZ), maximum phosphorus concentration (MAXP), and maximum nitrogen concentration (MAXN), (2) the local variables of distance to nearest AG patch (DISTAG), mean nearest neighborhood distances between WW patches (WWMNN-1k), and (3) the regional variables of percentage composition of the largest woody wetland patch (WWLPI-10k) and AG patch density (AGPD-10k) (Fig. 2). Mantel tests suggested that parasite abundances were not being significantly influenced by the spatial locations of our wetland study sites (all Ps ≥ 0.05), and therefore, the geographic locations of wetlands were not considered in subsequent analyses.

The explanatory variables selected for inclusion in the best models for each variable subset are presented for mean abundance and richness of LARVAL and
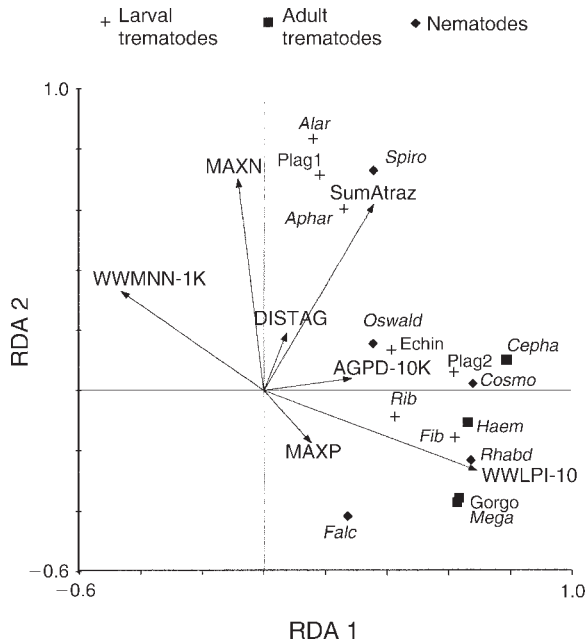
Fig. 2. Results of the redundancy analysis (based on the correlation matrix) displaying significant predictors of the mean parasite abundances per wetland. Significant variables ($P < 0.05$) were selected from the complete list presented in Table 1 using Monte Carlo permutations with a forward stepwise selection procedure (999 iterations). Displayed are the first and second extracted axes. Arrows represent environmental variables, which are defined in Table 1. Taxa are: *Alar*, *Alaria* sp.; *Aphar*, *Apharyngostrigea pipientis*; *Cepha*, *Cephalogonimus americanus*; *Cosmo*, *Cosmocercoides dukae*; Echin, echinostomatids; *Falc*, *Falcaustra* sp.; *Fib*, *Fibricola* sp.; Gorgo, gorgoderids; *Haem*, *Haematoloechus* sp.; *Mega*, *Megalodiscus temperatus*; *Oswald*, *Oswaldocruzia pipiens*; Plag1, unidentified plagiorchid1; Plag2, unidentified plagiorchid2; *Rhabd*, *Rhabdias ranae*; *Rib*, *Ribeiroia ondatrae*; *Spiro*, *Spiroxys* sp.

ADULT parasites, parasite diversity, and mean abundances for *R. ondatrae* and the echinostomatids in Table 3. In general, wetland and local variables were important for LARVAL abundances and richness in frog populations, as indicated by the higher R^2_{adj} values obtained for these subset models and by the variance partitioning analyses (Tables 3 and 4). The percentages of the invasive plants, *Phalaris* (%PHAL) and *Typha* (%TYPH), had the highest probabilities of being included in the best model for LARVAL richness. Nitrogen concentrations (MAXN) and WWMNN-1k were other important variables. A higher richness of LARVAL parasite taxa was associated with wetlands that had higher %PHAL, lower %TYPH, and lower MAXN (Table 5). The mean abundances of LARVAL infections were associated with higher SUMATRAZ and MAXP concentrations at the wetland level, as well as a greater percentage composition of FOR habitats (FORLD-1k) and smaller mean sizes of OW habitats (OWMPS-1k) at the local level (Tables 3 and 5).

Local and regional variables were most important for ADULT richness and abundances (Tables 3 and 4).

Greater taxa richness was detected at wetlands in landscapes with larger WW patches within 10 km (e.g., higher WWLPI-10k), higher densities of forest patches within 1 km (FORPD-1k), and located at greater distances from residential development (DISTRLD) (Tables 3 and 5). The largest patch index of woody wetland habitats at 10 km (WWLPI-10k) was the most important variable for ADULT abundances, as it was included in all competing overall models ($w_+ = 1$; Table 5), and this variable alone accounted for ~60% of the variance in ADULT mean abundances (R^2_{adj}, regional model alone; Table 4). Higher WWLPI-10k were associated with higher ADULT abundances (Table 5).

Diversities of the parasite communities were largely influenced by local landscape factors and to a lesser degree by wetland characteristics (Table 3). Overall, the average distances between the woody wetland patches at the local scale (WWMNN-1k) were the most important factor ($w_+ = 0.97$; Table 5), with higher parasite diversity being found at wetlands with shorter distances between the woody wetland patches in the surrounding 1-km landscapes. Density of forest patches (FORPD-1k), and amounts of agricultural (AGLD-1k), and forest land (FORLD-1k) at the local scale were other important variables (Tables 3 and 5).

Landscape variables measured at the regional spatial extent were the best predictors of *R. ondatrae* abundances, although snail abundance (NPLAN), a wetland variable, was also important (Tables 3 and 5). Higher abundances of the parasite were found in frogs at wetlands surrounded by more AG and WW patches, and supporting higher NPLAN (Table 5). Echinostomatid abundances were largely influenced by local and wetland variables. The abundances of these parasites increased with the percentage of FOR (FORLD-1k) and smaller mean sizes of OW patches (OWMPS-1k) at the local level, as well as with greater distances to the nearest WW (DISTWW), higher sum concentrations of naphthalene and phenanthrene (SUMPAH), and higher plant richness (VEGS) (Table 5).

DISCUSSION

Our study revealed that both wetland and landscape factors are important determinants of leopard frog parasite communities, although the responses to wetland and landscape factors found for LARVAL and ADULT parasite taxa suggest that processes operating at different scales may be influencing different aspects of parasite transmission dynamics. In particular, we found evidence to suggest that agricultural land use may cause two opposing responses in leopard frog parasites. Alteration of the landscape, and the loss of critical key habitats that support host populations, may lead to declines in parasite presence and abundance in frogs, whereas the addition of agricultural contaminants to wetlands may lead to increases in abundances of certain parasite species. Such dual responses to environmental change might be predicted for parasites with complex

TABLE 3. Explanatory variables included in the best models for each variable subset at four scales: wetland, local (1 km), regional (10 km), and overall.

Dependent variable and model†	Explanatory variables	k‡	AIC_c	R^2_{adj}
A) LARVAL				
Abundance				
Wetland	SUMATRAZ, MAXP	3	127.20	0.48
Local	FORLD-1k, OWMPS-1k	3	125.40	0.53
Regional	OWMPS-10k	2	130.78	0.25
Overall	SUMATRAZ, FORLD-1k, OWMPS-1k	4	110.59	0.83
Richness				
Wetland	%PHAL, %TYPHA, MAXN	4	65.57	0.66
Local	DISTAG, DISTRLD, AGLD-1k	4	72.16	0.51
Regional	OWMPS-10k, PWLD-10k	4	72.69	0.38
Overall	%TYPHA, %PHAL, MAXN	4	65.57	0.66
B) ADULT				
Abundance				
Wetland	NPLAN	2	70.78	0.14
Local	DISTFOR	2	72.56	0.05
Regional	WWLPI-10k	2	51.90	0.70
Overall	WWLPI-10k	2	51.90	0.70
Richness				
Wetland	NPLAN, %TYPHA	3	84.92	0.26
Local	DISTRLD, FORPD-1k	3	80.35	0.42
Regional	WWLPI-10k	2	80.10	0.33
Overall	WWLPI-10k, FORPD-1k, DISTRLD	4	70.52	0.73
C) Shannon *H'*				
Wetland	%TYPHA, MAXN	3	28.43	0.33
Local	FORPD-1k, WWMNN-1k, AGLD-1k	4	25.87	0.53
Regional	WWLPI-10k	2	31.53	0.07
Overall	WWMNN-1k, AGLD-1k, FORPD-1k	4	25.87	0.53
D) *Ribeiroia* abundance				
Wetland	NPLAN	2	77.59	0.13
Local	DISTWW, DISTAG	3	70.61	0.50
Regional	WWPD10k, AGPD-10k, OWMPS-10k	4	43.32	0.91
Overall	AGPD-10k, NPLAN, WWPD-10k	4	42.92	0.91
E) Echinostomatid abundance				
Wetland	VEGS, SUMPAH	3	104.14	0.43
Local	FORLD-1k, OWMPS-1k	3	102.46	0.49
Regional	AGLD-10K	2	108.09	0.17
Overall	FORLD-1k, OWMPS-1k, MAXP	4	101.03	0.61

Notes: These models were used in the variance partitioning analyses. ADULT refers to both adult trematodes and adult nematodes. LARVAL is equivalent to LTREM in the text, and both designations include only larval trematodes.

† Models were built for each separate spatial extent considered in the analysis and selected variables from the subsets listed in Table 1; the overall model considered the complete list of variables simultaneously.

‡ Number of parameters.

life cycles (e.g., Lafferty and Holt 2003), and are consistent with those reported for frog parasites in other regions (Koprivnikar et al. 2006, King et al. 2007, 2010, McKenzie 2007). Indeed, in a related analysis, Rohr et al. (2008b) showed that the positive effects of atrazine exposure on amphibian larval trematode abundance depended on the amount of suitable host habitat in the surrounding landscape.

In general, both LARVAL and ADULT parasites seemed to be responding similarly to wetland and landscape factors, though the specific parameters most important for these different types of parasites varied. Also, the spatial extent explaining the highest amount of variation in LARVAL and ADULT communities and

overall abundances differed between the groups. Wetland and local factors were particularly important for LARVAL taxa, whereas local and regional factors were most important for ADULT parasites (Table 4). This was a surprising observation because the definitive hosts of the LARVAL parasites, birds and mammals, presumably have greater dispersal abilities than frogs, which are the definitive hosts of the ADULT taxa detected in our study. Therefore, it might be expected that LARVAL parasites would be more influenced by regional variables than ADULT parasites. Moreover, previous investigations suggested wetland characteristics were the predominant determinants of the distributions of ADULT frog parasite taxa, although those studies did

TABLE 4. Results of variance partitioning analyses quantifying the amount of variation (adjusted R^2) unique to, or shared by, wetland, local, and regional variable subsets for parasites classified as larval trematodes and adult trematodes and nematodes.

Variance component	LARVAL		ADULT	
	Richness	Mean abundance	Richness	Mean abundance
Wetland (W)‡	0.166*	0.291†	−0.012	−0.001
Local (L)‡	0.065	0.278†	0.243**	0.026
Regional (R)‡	0.112	−0.001	0.263†	0.606†
WL	0.198	0.024	0.152	0.016
LR	−0.028	0.089	−0.049	−0.032
WR	0.023	0.026	0.040	0.085
WLR	0.270	0.136	0.078	0.039
Residual	0.194	0.156	0.285	0.260

Notes: Models used in each variable subset were those identified as the best based on minimum AIC$_c$ values in Table 3. ADULT refers to both adult trematodes and adult nematodes. LARVAL is equivalent to LTREM in the text, and both designations include only larval trematodes.

* $P < 0.05$; ** $P < 0.01$; † $P < 0.005$.

‡ Probability values can only be calculated for these components.

not quantify the relative contributions of wetland vs. landscape factors (Aho 1990, Muzzall 1991, Koprivnikar et al. 2006, McKenzie 2007). The stronger association between ADULT and regional variables observed in our study may reflect the negative effects that broad landscape changes have on frog populations (Knutson et al. 1999, Trenham et al. 2003, Price et al. 2004, Mazerolle et al. 2005), and supports the idea that broad land use changes can significantly affect disease processes in remnant habitat patches (Taylor and Merriam 1996, Collinge et al. 2005).

Of the landscape factors examined, we found that leopard frog parasite abundances were most consistently related to forest, woody wetland, and open water components of the surrounding landscapes. In particular, higher parasite abundances tended to be found in frogs from landscapes composed of more forest and woody wetland habitats, and with small to intermediate sized patches of open water. Metrics that are associated with the connectivity, such as mean nearest neighborhood distances, and aggregation, such as density and largest patch index, were also generally more important than the percentage composition of the habitats in the landscapes surrounding wetlands. These patterns were consistent across the local and regional spatial scales examined and for both LARVAL and ADULT parasites, and probably reveal the importance of forest, woody wetland, and open water habitats to the definitive hosts of the parasites. For instance, fewer waterbirds visit wetlands in areas that are heavily impacted by agriculture (Leitch and Kaminski 1985, Bethke and Nudds 1995, Krapu et al. 1997) because they prefer seasonal wetlands for breeding and foraging over semipermanent or permanent wetlands (Talent et al. 1982, Merendino et al. 1995). In agricultural areas, most seasonal wetlands are drained and converted to cropland; as a consequence, waterbirds settle less frequently in the seasonal wetlands remaining (Krapu et al. 1997). Herons, hawks, and small mammals, other definitive hosts that may be particularly important in the

transmission of *R. ondatrae* (Johnson et al. 2004), also are likely to be less abundant in regions dominated by agricultural land because of their dependence on forested or woody wetland habitats (Gibbs et al. 1987, Nupp and Swihart 2000). Frogs, the definitive hosts of ADULT taxa, are also known to be highly dependent on the availability of forest habitats (Knutson et al. 1999, Trenham et al. 2003, Mazerolle et al. 2005). Thus, the patterns observed in the parasite communities of metamorphic frogs may, in part, indirectly reflect a negative response to agriculture as a result of the loss of forest, woody wetland, and open water habitats and changes in the connectivity of these natural habitats. King et al. (2007, 2010) found a similar pattern of reduced parasitism in frogs found at wetlands in heavily cultivated landscapes in Quebec, Canada.

In addition to the likelihood that frog parasite distributions are influenced by definitive host behavior and their responses to environmental change, we found evidence to suggest that agricultural land use may also directly affect frog parasite communities by influencing transmission dynamics within wetlands. In particular, the sum concentrations of atrazine and desethylatrazine were significantly related to the LARVAL abundances we observed in our study sites. In fact, in our analyses here and in Rohr et al. (2008b), this variable alone explained the greatest percentage of the variance in the LARVAL abundance data. Atrazine is probably the most widely used herbicide in the United States, and it is commonly found in ground and surface waters (Rohr and McCoy 2010). It has been shown to be teratogenic and immunosuppressive in leopard frogs (Christin et al. 2003, Hayes et al. 2003, Rohr and McCoy 2010), but it is not directly lethal to snails at ecologically relevant concentrations (Rohr et al. 2008a). In addition, Kiesecker (2002) and Rohr et al. (2008a) found that tadpoles exposed to atrazine in the laboratory were more susceptible to larval trematode infection. Therefore, it is possible that the positive association between atrazine concentrations and LARVAL abundances detected in

TABLE 5. Weighted coefficients and standard errors for the explanatory variables that had probabilities (w_+) greater than 0.20 of being included in the best model, based on the overall models with $\Delta AIC_c \leq 4.01$.

Dependent variable	Explanatory variable	No. models	w_+	Coefficient	SE
A) LARVAL					
Abundance	Intercept	3		0.13	2.660
	SUMATRAZ		1	31.73	7.088
	FORLD-1k		0.88	0.77	0.127
	OWMPS-1k		0.88	−0.70	0.125
Richness	Intercept	11		5.79	1.340
	%PHAL		0.87	0.042	0.012
	%TYPHA		0.63	−0.024	0.008
	WWMNN-1k		0.35	−0.004	0.002
	MAXN		0.33	−0.147	0.055
B) ADULT					
Abundance	Intercept	37		0.59	0.672
	WWLPI-10k		1	3.43	0.559
	DISTFOR		0.28	−0.003	0.001
Richness	Intercept	1		−1.75	1.025
	DISTRLD			0.002	0.0006
	FORPD-1k			0.32	0.079
	WWLPI-10k			3.3	0.784
C) Shannon H'	Intercept	33		1.25	0.516
	WWMNN-1k		0.97	−0.002	0.0005
	AGLD-1k		0.31	0.016	0.008
	FORPD-1k		0.30	0.048	0.025
	FORLD-1k		0.29	−0.027	0.014
	OWMPS-1k		0.21	−0.027	0.014
D) *Ribeiroia* abundance	Intercept	17		−1.9	0.714
	AGPD-10k		1	0.31	0.075
	NPLAN		0.80	0.01	0.004
	WWPD-10k		0.45	0.94	0.412
E) Echinostomatid abundance	Intercept	19		4.75	5.025
	FORLD-1k		0.40	0.44	0.121
	OWMPS-1k		0.40	−0.39	0.107
	DISTWW		0.32	0.005	0.002
	SUMPAH		0.29	4.29	1.714
	VEGS		0.26	0.34	0.119
	DISTRLD		0.22	0.005	0.001

Notes: When no w_+ are displayed, there were no models that competed with the most parsimonious model (e.g., there were no other models with $\Delta AIC_c \leq 4.01$). ADULT refers to both adult trematodes and adult nematodes. LARVAL is equivalent to LTREM in the text, and both designations include only larval trematodes.

this study is indicative of the immunosuppressive effects of atrazine, and may suggest that tadpoles developing in atrazine-contaminated wetlands are at greater risk of larval trematode infection (Rohr et al. 2008b).

King et al. (2007) also studied parasite abundances in leopard frogs in relation to atrazine concentrations in wetlands, but did not report a positive effect of atrazine on parasite abundances. In fact, King et al. (2007) found their most depauperate parasite communities in wetlands where their highest atrazine concentrations were detected. This lack of congruence between studies may be related to regional differences in the amounts of land in cultivation. For instance, the wetlands studied in Quebec, Canada by King et al. (2007) had less forest available within 100- and 500-m spatial extents (2% and 7.6%, respectively), compared to the wetlands we studied in Minnesota (18% and 13%, respectively). It may be that the presence of higher amounts of forest around the

wetlands in Minnesota support higher densities of and more frequent visits by definitive hosts, such that inputs of parasite infective stages within these wetlands are higher than those studied by King et al. (2007), and therefore, the effects of atrazine on infection rates in amphibians are more evident. In support of this suggestion, Rohr et al. (2008b) found a significant interaction effect between atrazine concentrations and the combined densities of suitable habitat patches (e.g., forest and wetland) around wetlands on larval trematode abundances, with the effect of atrazine being stronger where there was more suitable habitat for hosts. Likewise, we detected high shared variances among the wetland, local, and regional variable subsets for the LARVAL parasites in the current analysis (Table 4), suggesting that interactions among wetland and landscape factors are important.

We also found a positive association between LARVAL abundances and phosphorous concentrations, consistent with the hypothesis that increased nutrients will lead to increased snail densities and LTREM infections in amphibians (Johnson et al. 2007, Rohr et al. 2008b). However, we did not detect a positive relationship between nitrate concentrations and parasite abundances, as predicted (e.g., McKenzie and Townsend 2007, Johnson et al. 2010); and instead, a negative relationship between nitrate concentrations and LARVAL richness was detected. This latter relationship might be related to the influence of nutrient enrichment on wetland vegetation. Nutrient enrichment has been shown to promote the growth of invasive wetland plant species, such as *Typha* spp. and *Phalaris* spp. (Verhoeven et al. 1996, Maurer and Zedler 2002), and high nitrogen concentrations may be particularly important for the growth of these plants in midwestern wetlands (Craft et al. 2007). Composition of the wetland vegetation community is likely important for amphibian parasite abundance and diversity because it may influence snail richness and abundance (Negovetich and Esch 2007), attract definitive hosts to wetlands (Kaminski and Prince 1981, Elmberg et al. 1993), or influence the likelihood of contact between parasite infective stages and tadpoles (Murphy et al. 2000). Interestingly, in our study, %TYPHA was negatively associated with LARVAL richness, whereas %PHAL was positively associated with LARVAL richness. It is not clear if these opposing associations are related to inherent properties of the particular invasive plants or the overall habitat changes that might accompany their invasions. Our observations suggest there is a need to better understand the potential interactions among nutrient concentrations, wetland vegetation, snail abundance, and trematode infection prevalence.

Factors that are predictive of *R. ondatrae* presence and abundance at a wetland are of interest because of the link between infections with this parasite and limb malformations (Johnson et al. 1999, 2002). It has been suggested that land use changes and eutrophication of water bodies associated with agriculture and cattle production in the western United States have allowed *R. ondatrae* to proliferate and possibly increase its range because of the increased food resources available for its snail host in such altered environments (Johnson et al. 2002, 2007, 2010). Kiesecker (2002) also found evidence to suggest that amphibians that developed in wetlands that received pesticide run-off from agricultural fields had a higher risk of developing a *R. ondatrae*-induced malformation than those in nonagricultural wetlands. In our study, *R. ondatrae* abundance was positively associated with *Planorbella* abundance (Tables 3 and 5), but it was not significantly related to SUMATRAZ or MAXP (see also Rohr et al. 2008b). On the other hand, we did detect strong positive associations between *R. ondatrae* abundances and patch densities of agricultural land and woody wetland patches at the regional scale,

suggesting that the availability of definitive host habitats, and specifically, woody wetland habitats, is important for *R. ondatrae* abundance, and that perhaps a highly fragmented landscape within an agricultural matrix somehow contributes to its success. It is also possible that the effects of nutrient inputs and contaminants on *R. ondatrae* infections in amphibians were not detected in our study because it was a fairly rare parasite in our study region. An examination of water bodies along a gradient of nutrient conditions in landscapes with varying degrees of wooded habitat where *R. ondatrae* is more common may help further define the environmental factors related to *R. ondatrae* occurrence in Minnesota.

Echinostomatids are of interest not only because they were so predominant, but also because of their potential to cause mortality in larval amphibians (Schotthoefer et al. 2003, Holland et al. 2007, Szuroczki and Richardson 2009). We found little evidence to suggest that echinostomatid abundances in frogs are positively influenced by residential land use, as suggested by Skelly et al. (2006). Higher abundances were found at wetlands that were farther from the nearest RLD patch in our study (Table 5). In contrast, echinostomatids appeared to be responding similarly to forest and open water habitats as the other parasite taxa detected in our study, and to be negatively influenced by landscapes dominated by agricultural land. However, the positive association between echinostomatid abundances and the sum concentrations of phenanthrene and naphthalene (SUMPAH) does suggest a possible association with suburban or urban areas. Phenanthrene and naphthalene are polycyclic aromatic hydrocarbons, which are by-products of the incomplete combustion of fossil fuels, coal and oil burning, wood combustion, or coke and oil shale plants, activities that tend to increase with urbanization.

Although our study supports the hypothesis that anthropogenic activities influence parasite transmission dynamics, how parasite infection rates should be interpreted with regard to the health of frog populations must be considered cautiously. Frog populations located in wetlands that have been less impacted by human activities have been reported to harbor more parasites, suggesting that high parasite richness or abundance may be indicative of healthier ecosystems (Koprivnikar et al. 2006, King et al. 2007, 2010). This idea was supported in our study by observations that the parasites that mature in frogs (e.g., ADULTs) were positively associated with wetlands in landscapes with more woody wetland and forested habitats. Conversely, abundances of the LARVAL parasites were positively related to atrazine and phosphorous concentrations, and thus their abundances may be more suggestive of anthropogenetic activities related to inputs of agrochemicals into wetlands. The parasite taxa that were most influenced by atrazine, in particular, were *Alaria* sp., one of the unidentified Plagiorchids, and *Apharyngostrigea pipientis* (Fig. 2). Whether there is something unique about the

life cycles of these parasites that leads to such elevated abundances in contaminated wetlands is not known. Moreover, it is not clear why ADULT parasites were not as strongly influenced by nutrients or contaminants as the LARVAL parasites, given that many of their life cycles also involve snail intermediate hosts (Table 2). Our data suggest that the differences between LARVAL and ADULT parasites may relate to variation in the degree to which the definitive hosts are dependent on the availability of forest and woody wetland habitats in surrounding landscapes. Despite an increase in snail abundance that might occur in response to nutrient inputs, the ultimate source of ADULT infections in the snail population is frogs. If more nutrients and contaminants are associated with wetlands surrounded by more agricultural land and less forested or woody wetland habitats, frog populations would be expected to be smaller and thus the inputs of infective stages from frogs into wetlands would also be lower in these landscapes. The definitive hosts of parasites, birds and mammals, are also dependent on suitable habitats in the surrounding landscape; however, their greater dispersal abilities may ensure that some individuals visit and deposit eggs that subsequently become LARVAL infective stages even in those wetlands that have less suitable habitat in the surrounding landscapes.

In summary, the distributions and abundances of parasites in metamorphic leopard frog populations in Minnesota were related to landscape structure and wetland contamination associated with agricultural land use. The losses of forest fragments, large woody wetland fragments, and small open water patches at local and regional scales, in particular, were correlated with fewer parasite species and lower abundances in frogs, perhaps reflecting fewer definitive hosts of the parasites in these landscapes. Conversely, the contamination of wetlands by agrochemicals may act to increase certain parasite abundances via other mechanisms, such as by immuno-suppressing frogs and/or snails, reducing cover, or elevating snail populations (Murphy et al. 2000, Sandland and Carmosini 2006, Rohr et al. 2008*b*, Szuroczki and Richardson 2009, Johnson et al. 2010). Our results suggest that different types of anthropogenic change may influence parasitism in different ways, with infections by ADULT parasites being more related to broad landscape changes and LARVAL parasites more responsive to alterations in wetland quality. However, the evidence of possible interactions between and among wetland and landscape factors suggests that there is a need to better understand the roles of definitive host densities and visitation rates as they relate to landscape and wetland-specific characteristics so that appropriately scaled intervention strategies can be implemented to reduce adverse impacts of parasitism on anuran populations. At present, the spatial scales identified as most important for larval trematodes, the parasites of greatest current concern, suggest that management strategies that aim to reduce inputs of agrochemicals into wetlands, may have the greatest success in limiting these infections.

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APPENDIX A

Locations of wetland study sites included in the analysis (*Ecological Archives* A021-058-A1).

APPENDIX B

A table showing land cover classifications used in quantifying landscape structure at the 1-km and 10-km spatial extents examined, as they corresponded to the 1990s National Land Cover data (NLCD) classes (*Ecological Archives* A021-058-A2).

APPENDIX C

Descriptive statistics for the explanatory variables for the 18 surveyed wetlands included in the analyses (*Ecological Archives* A021-058-A3).