

AGRICULTURAL EFFECTS ON AMPHIBIAN PARASITISM: IMPORTANCE OF GENERAL HABITAT PERTURBATIONS AND PARASITE LIFE CYCLES

Janet Koprivnikar^{1,2} and Julia C. Redfern¹

¹ Department of Biology, Brandon University, John R. Brodie Science Centre, 270 18th Street, Brandon, Manitoba R7A 6A9, Canada

² Corresponding author (email: koprivnikarj@brandonu.ca)

ABSTRACT: Agricultural activity can alter host-parasite interactions through associated contaminants and habitat perturbations. It is critical to determine whether agricultural effects are widespread or limited to specific types of agriculture. We examined influences of soybean agriculture on trematode parasitism of larval amphibians (grey tree frogs; *Hyla versicolor*) to assess the potential effects of a commonly applied pesticide (glyphosate) and landscape factors relative to previous field studies focusing on the herbicide atrazine. Overall, trematode parasite infection did not differ between soybean-adjacent and nonagricultural ponds (87.7% and 72.6% mean infection, respectively). However, host-generalist echinostome species were more common in tadpoles from soybean-associated ponds (86.3% mean infection versus 36.2% in nonagricultural ponds) as well as sites with large or short average distances to forest cover and roads, respectively. In contrast, the occurrence of a host-specialist (*Alaria* sp.) group was greater in nonagricultural ponds (50.3% mean infection versus 9.8% in soybean-associated ponds) and increased with shorter distances to the closest forest patch and smaller average forest distance. Because glyphosate was not detected at any site and landscape influences were parasite-specific, we suggest that agriculture may have broad effects on wildlife diseases through habitat alterations that affect pathogen transmission via host habitat suitability. Notably, nonagricultural ponds had a lower mean distance to the nearest forest patch and lower mean forest distance compared with soybean-adjacent ponds. As a result, we emphasize the need for wider investigations of habitat perturbations generally associated with agriculture for host-pathogen interactions, and consequently, wildlife conservation and management strategies.

Key words: Agriculture, amphibian, disease, habitat, landscape, parasite.

INTRODUCTION

Numerous diseases, particularly those caused by emerging infections, have now been linked to anthropogenically driven environmental changes including urbanization, habitat fragmentation, deforestation, altered food webs, climate change, and contaminants (e.g., Harvell et al., 2002; Bradley and Altizer, 2007). Reports of diseases in freshwater taxa are increasing and of special concern (Johnson and Paull, 2011), playing a particularly important role in the global decline of amphibian populations (e.g., Daszak et al., 2003). The effect of agricultural activities on wildlife diseases has received much attention. These are often associated with altered landscapes and various contaminants, which in turn affect host-pathogen interactions. In particular, components of agricultural runoff have significant effects

on amphibian disease in the field and laboratory by increasing host susceptibility to infection via immunosuppression (e.g., Kiesecker, 2002; Christin et al., 2003). Runoff constituents have also been shown to facilitate parasite development within hosts and increase the density of intermediate hosts needed for the transmission of parasites with complex life cycles (e.g., Johnson and Chase, 2004; Rohr et al., 2008a, b).

However, agricultural activities can affect disease patterns simply by altering land use, similar to urbanization and deforestation, excluding contaminant-driven effects (e.g., McKenzie, 2007). Urbanization often leads to biodiversity loss and changing species composition and thus potentially affecting parasite transmission (Bradley and Altizer, 2007; Lehrer et al., 2010). Roads associated with urbanization can affect disease patterns by facilitating

the movement of pathogens or their hosts or modifying the environment to provide suitable habitat for either (Patz et al., 2000; Molyneux, 2003). Deforestation has enormous effects on entire ecosystems and can also affect the transmission of both human and wildlife diseases (e.g., Killilea et al., 2008; Sehgal, 2010). Significantly, the effects of deforestation on many of these diseases are largely mediated through influences on their arthropod vectors (Sehgal, 2010). It is, therefore, critical to consider whether landscape/habitat alterations associated with agricultural activities are having broad and widespread effects on wildlife diseases in addition to contaminant-driven effects. Such knowledge is crucial for the conservation and management of wildlife and especially relevant for species and populations that are endangered or at risk, including many amphibians. Notably, fungal pathogens, such as *Batrachochytrium dendrobatidis* (*Bd*), are having devastating effects on amphibians worldwide, but there is an increasing recognition that other pathogens, including trematode parasites, may also have effects (Johnson and McKenzie, 2008; Szuroczi and Richardson, 2009).

Recent field studies have highlighted the importance of considering wetland and landscape aspects, in addition to agricultural contaminants, with respect to amphibian parasitism (e.g., Koprivnikar et al., 2006; McKenzie, 2007; King et al., 2008; Schotthoefler et al., 2011). However, most previous field studies examining amphibian parasitism in relation to environmental factors focused on the presence of the herbicide atrazine either by choice or by virtue of its common occurrence (e.g., Koprivnikar et al., 2006; Rohr et al., 2008b; King et al., 2010). In addition, atrazine has documented effects on amphibian susceptibility to parasitism (Kiesecker, 2002; Rohr and McCoy, 2010). It is not clear whether habitat alterations associated with agricultural activities have broad effects on amphibian parasitism

given that these have tended to be confounded with atrazine presence in previous field studies. For instance, although atrazine was the best predictor of larval amphibian trematode infection among wetlands in Minnesota, USA, that was context-dependent because there was a significant interaction between atrazine and the number of habitat patches that could conceivably support visitation by final hosts (Rohr et al., 2008b). It is, therefore, necessary to examine whether such landscape effects might still occur with agricultural activities associated with other pesticides (i.e., not atrazine).

Additionally, not all environmental perturbations affect host-pathogen interactions in the same way, and many can actually result in decreased host infection and even pathogen elimination (Lafferty, 1997; Hudson et al., 2006). Parasites with specialized or complex life cycles are anticipated to be especially sensitive to environmental disturbances (Lafferty and Holt, 2003; Johnson and Paull, 2011) and should exhibit reductions if habitat perturbations, such as deforestation or fragmentation, decrease the diversity of potential intermediate and definitive hosts (Hudson et al., 1998; Marcogliese, 2005). There is evidence for such species-specific effects with respect to amphibian parasites (e.g., Koprivnikar et al., 2006; King et al., 2007), but that requires further study.

We examined the influence of agricultural activity in the form of soybean crops on trematode parasite infections of larval amphibians. Our purpose was to examine whether this specific type of agriculture was related to amphibian parasitism as well as landscape factors (forests and roads), previously shown to have influences by affecting host-habitat suitability. Previous field studies have found a generally positive association between amphibian helminth infections and agricultural activity (e.g., Koprivnikar et al., 2006; Rohr et al., 2008b; King et al., 2010); but again, these studies mainly focused on the presence of the herbicide atrazine

(typically associated with corn crops). By focusing on ponds adjacent to soybean fields, a crop for which atrazine is not applied (Pawlak et al., 1987), we decreased the chances that landscape factors were confounded with the presence of atrazine. Additionally, the glyphosate-based compounds (e.g., Roundup®, St. Louis, Missouri, USA) most often associated with soybean crops are now the most widely applied herbicides in the world (Struger et al., 2008) and have documented effects on aquatic vertebrate mortality and susceptibility to parasitism in laboratory experiments (e.g., Rohr et al., 2008a; Kelly et al., 2010).

MATERIALS AND METHODS

Larval amphibian sampling

We surveyed 10 permanent ponds in July 2010 with five of them directly adjacent to soybean fields and five in nonagricultural settings (nearest field/pasture >75 m away). These ponds were randomly chosen from a larger set of 18 ponds (nine soybean-adjacent and nine nonagricultural) identified as possible sampling sites that year. Previous fieldwork in this area had established that the grey tree frog (*Hyla versicolor*) larvae were most likely to be present, thus only those tadpoles were collected. A total of 157 grey tree frog tadpoles were collected (13–22/site; Table 1), with 79 from soybean ponds and 78 from nonagricultural ponds. Each site was sampled once, with tadpoles caught with sweep nets, taken to the laboratory, euthanized by immersion in buffered MS-222 (tricaine methanesulfonate; Sigma Aldrich, Oakville, Canada), and preserved in ethanol for later dissection and examination of trematode infection. Trematodes were

identified using representative parasite specimens and guides by Schell (1970, 1985). Representative parasite specimens were removed from the first two tadpoles in each pond infected with a particular trematode type (e.g., mesocercariae in coelom). It was then assumed that all other mesocercariae in tadpoles from that pond were of the same trematode genus. Protocols adhered to guidelines from the Canadian Council on Animal Care and were approved by the Brandon University animal care committee (2010R02).

Site water and habitat analysis

At the time of tadpole collection, 2 L of water were taken from each sampled pond, refrigerated, and analyzed within 1 wk for glyphosate, nitrite, and phosphorus by the Laboratory Services Division at the University of Guelph (Guelph, Ontario). Because grey tree frogs primarily use highly wooded habitats for breeding (Ralin, 1968), we found that three of our five sampled, nonagricultural sites were not noted on digital Ontario, Canada, base maps, likely because of their limited aerial visibility, precluding the use of analysis with standard geographic information system methods. Therefore, we used tools available using Google Earth® (Mountain View, California, USA) to measure the following habitat variables: mean road distance (using the closest paved and unpaved roads to the north, south, east, and west of the pond), distance to the closest road (paved or unpaved in any direction), mean forest distance (using the closest forest patches to the north, south, east, and west of the pond), and distance to the closest forest patch (any direction). Data for these landscape variables were tabulated for all 18 ponds (Table 2).

Statistical analysis

We calculated the prevalence of infection for each of four trematode species in each pond (percentage of tadpoles infected) based

TABLE 1. Trematode parasite infection measures of grey tree frog *Hyla versicolor* tadpoles from sampled ponds. Infection measures represent proportion (%) of parasitized tadpoles for each site.

Pond No.	1	2	3	4	5	6	7	8	9	10
No. of tadpoles sampled	22	14	13	14	16	15	16	16	15	16
Overall trematode infection (%)	100	64	93	100	81	40	100	93	80	50
Echinostome infection (%)	100	64	93	93	81	13	19	38	80	31
<i>Alaria</i> sp. infection (%)	14	0	23	0	13	40	44	93	27	50
Skin cyst sp. infection (%)	5	0	0	21	6	0	100	0	73	0
<i>Ribeiroia ondatrae</i> infection (%)	0	0	0	7	0	0	13	0	13	0
No. of trematode species	4 ^a	2	2	3	3	2	4	2	4	2

^a Rare trematodes in the category of “other” are not shown here but are included in the count of trematode species.

TABLE 2. Habitat measures of ponds sampled (ponds 1–10) and unsampled (ponds 11–18) for grey tree frog *Hyla versicolor* tadpoles.

Pond No.	Soybean field	Glyphosate	Nitrate (mg/L)	Phosphorus (mg/L)	Distance to closest road (m)	Average road distance (m)	Distance to closest forest (m)	Average forest distance (m)	Location
1	Y ^a	N	0.107	<0.05	49.5	549.3	58.4	546.3	43°13'6.95"N, 81°0'20.16"W
2	Y	N	<0.006	<0.05	16.3	438.1	147.1	482.9	43°13'5.17"N, 81°0'15.64"W
3	Y	N	<0.006	<0.05	26.6	433.1	33.3	939.4	43°28'21.91"N, 80°14'24.23"W
4	Y	N	<0.006	<0.05	30.7	392.4	151.3	720.5	43°28'19.03"N, 80°14'26.17"W
5	Y	N	<0.006	<0.05	24.2	796.8	23.9	423.5	43°33'40.64"N, 80°8'11.84"W
6	N	N	<0.006	<0.05	81.9	870.6	29.1	63.7	43°27'35.78"N, 80°13'13.91"W
7	N	N	<0.006	<0.05	17.4	789.7	10.6	32.5	43°27'44.78"N, 80°13'12.38"W
8	N	N	<0.006	<0.05	20.2	722.4	4	35.8	43°29'53.50"N, 80°7'14.40"W
9	N	N	<0.006	<0.05	25.1	613.4	48.8	80.4	43°31'36.71"N, 80°5'16.20"W
10	N	N	<0.006	<0.05	50.3	736.9	9.3	20.3	43°30'47.63"N, 80°8'4.93"W
11	Y	n/a	n/a	n/a	81.4	466.4	38.2	594.2	43°14'2.75"N, 81°0'41.63"W
12	Y	n/a	n/a	n/a	102.9	1,110.8	38.4	797.6	43°13'38.57"N, 81°1'16.26"W
13	N	n/a	n/a	n/a	93.4	850.3	39.8	72.1	43°11'51.48"N, 81°0'21.89"W
14	Y	n/a	n/a	n/a	198.6	1,060.7	51.2	1,298.7	43°9'52.94"N, 80°57'20.23"W
15	N	n/a	n/a	n/a	99.7	922.1	55.6	737.9	43°14'34.20"N, 80°56'44.13"W
16	N	n/a	n/a	n/a	25	370	9.8	391.3	43°33'43.67"N, 80°8'15.92"W
17	N	n/a	n/a	n/a	30.2	296.1	18.7	126.5	43°28'30.38"N, 80°11'11.87"W
18	Y	n/a	n/a	n/a	38.8	760.1	27.4	102.3	43°29'58.11"N, 80°9'4.42"W

^a Presence or absence of factors is denoted by Y (yes) or N (no), respectively, with n/a indicating no testing.

on those most common among sites (parasites excluded if not present at $\geq 30\%$ of the sites), as well as overall infection prevalence (any trematode species), and trematode species richness (number of species per site). The values for each pond were arcsine-transformed, with the exception of the log-transformed values for trematode species richness. We then used generalized linear-model procedure in SPSS 19.0 (IBM SPSS Inc., Chicago, Illinois, USA) with a linear-scale response to generate models predicting the prevalence of each trematode species, overall infection, and

species richness in ponds based on soybean presence, average road and forest distances, and closest road and forest distances. Water-based variables were not included because of their minimal variation among ponds (nine of 10 ponds were <0.006 mg/L for nitrate, and all were <0.05 for phosphorus). We tested for overall correlations among road and forest measures for the sampled ponds with Pearson's correlation tests. In addition, we conducted two separate one-way analysis of variance (ANOVA) tests to determine whether our habitat variables differed between the 10

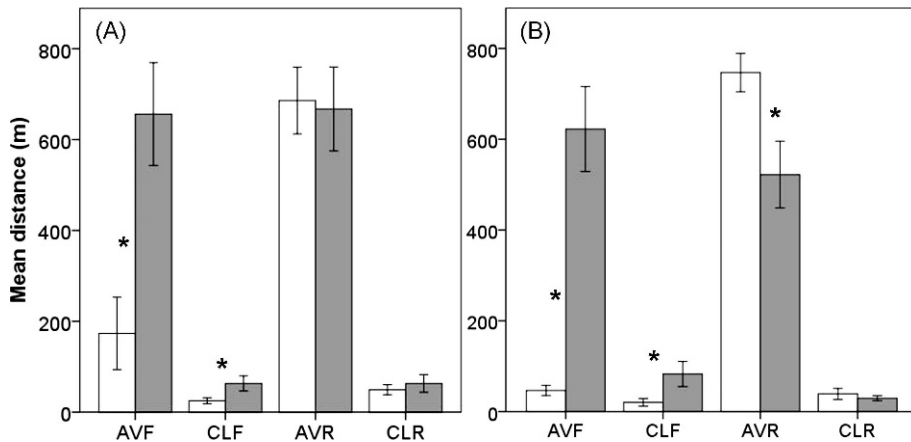


FIGURE 1. Mean habitat values (\pm SE) for ponds either adjacent to soybean fields (grey bars) or nonagricultural (white bars). Habitat values include (A) average distance to forest (AVF), distance to closest forest patch (CLF), average road distance (AVR), and distance to the closest road (CLR) for all 18 ponds, or (B) the 10 ponds sampled for grey tree frog *Hyla versicolor* tadpoles. Significant differences between the two pond types are noted with an asterisk.

sampled soybean and nonagricultural ponds as well as between these two pond types for the larger set of 18 ponds. All analyses were conducted using SPSS 19.0.

RESULTS

Host parasitism

Mean overall trematode infection across all 10 ponds was 80%, varying from 40% to 100% of tadpoles infected in each pond (Table 1). Echinostome cysts with a collar of spines were seen in the kidneys of 61% of all tadpoles (present at all 10 sites, 13–100% of tadpoles infected in each pond). *Alaria* sp. mesocercariae were seen in the coelom of 30% of all tadpoles (present at eight sites, 13–93% of tadpoles infected in those ponds). Skin cysts (likely *Glypthelmins* sp. owing to the stylet in the cysts examined) were noted as a single category and found in 21% of all tadpoles (present at five sites, 5–100% of tadpoles infected in those ponds). Unidentified cysts in tail musculature were observed for 2% of all tadpoles (present at two sites, 6–13% of tadpoles infected in those ponds), and cysts around/in the eyes identified as *Ribeiroia ondatrae* were observed in 3% of tadpoles (present at three sites, 7–13% of tadpoles infected in those ponds). Mean trematode

species richness was 2.7 (two to four trematode species present at each site).

Site water and landscape characteristics

Glyphosate was not detected in any of the sampled ponds, and all phosphorus levels were below 0.05 mg/L. Similarly, nitrate levels were below 0.006 mg/L across sampled sites with the exception of one soybean pond with a level of 0.0169 mg/L. The results of the one-way ANOVA for the 10 sampled ponds indicate statistically significant differences between soybean and nonagricultural ponds with respect to average forest distance ($F_{1,8}=37.409$, $P<0.001$), closest forest distance ($F_{1,8}=6.920$, $P=0.030$), and average road distance ($F_{1,8}=7.027$, $P=0.029$), but not closest road distance ($F_{1,8}=2.160$, $P=0.180$). Generally, the sampled ponds adjacent to soybean fields had a greater distance to the closest forest patch and greater average forest distance but smaller average road distance (Fig. 1). For the larger set of 18 ponds, those adjacent to soybean fields were significantly farther from the closest forest patch and had a significantly higher average forest distance ($F_{1,16}=4.593$, $P=0.048$) and had a significantly higher average forest distance ($F_{1,16}=12.111$, $P=0.003$) but did not differ

from nonagricultural ponds with respect to closest road distance ($F_{1,16}=0.390$, $P=0.541$) or average road distance ($F_{1,16}=0.024$, $P=0.879$). There was a significant, negative relationship between closest forest distance and average road distance ($r=-0.762$, $P=0.010$), with a similar result seen for average forest and road distances ($r=-0.790$, $P=0.007$).

Associations among parasitism and landscape measures

None of the models generated to predict overall trematode infection, prevalence of skin-cyst forming trematodes, prevalence of *R. ondatrae*, or trematode species richness in ponds were statistically significant. However, significant models were generated for prevalence of echinostome and *Alaria* sp. infections. The presence of soybean fields was a significant predictor of echinostome infection ($\chi^2=9.644$, $P=0.002$) because the prevalence was higher when soybean fields were present (Fig. 2). Average distances to forest patches and roads, respectively, were also significant predictors of echinostome prevalence ($\chi^2=9.64$, $P=0.002$; $\chi^2=6.828$, $P=0.009$) because infections increased with greater average distance to forest but decreased with larger average distance to roads (Fig. 3). Although the presence of soybean fields was also a significant predictor of *Alaria* sp. infection ($\chi^2=6.783$, $P=0.009$), the pattern was opposite to that for echinostomes because the prevalence of infection for *Alaria* sp. was higher in nonagricultural ponds. *Alaria* sp. infection was also predicted by average distance to forest ($\chi^2=4.936$, $P=0.026$), with a higher prevalence of infection as that distance decreased. A similar relationship was seen for distance to the closest patch of forest and *Alaria* sp. infection ($\chi^2=5.832$, $P=0.016$).

DISCUSSION

Although agricultural effects on amphibian parasitism can be mediated

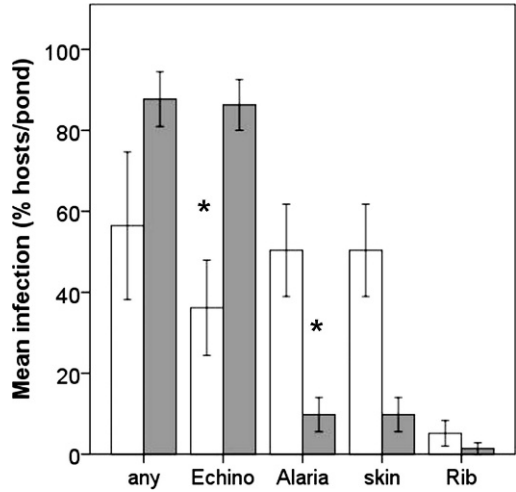


FIGURE 2. Mean infection values (\pm SE) for grey tree frog *Hyla versicolor* tadpoles in ponds either adjacent to soybean fields (grey bars) or nonagricultural (white bars). Infection values include hosts with any trematode infection (any), echinostome infection (Echino), *Alaria* sp. infection (Alaria), infection by a skin-encysting species (skin), and infection with *Ribeiroia ondatrae* (Rib). Statistically significant differences between the two pond types are noted with an asterisk.

through contaminant effects, our results indicate that general aspects of agricultural activities (i.e., habitat modifications) may also play a major role. The presence of soybean agriculture had no effect on overall prevalence of trematode infection in larval grey tree frogs; however, tadpoles from ponds adjacent to soybean fields had a significantly higher prevalence of echinostome trematode-species infection, regardless of there being no herbicide glyphosate detected at any site. In contrast, the prevalence of *Alaria* sp. trematodes was greater in nonagricultural ponds. Infection by both echinostomes and *Alaria* sp. was also dependent on the closeness of forests and roads. The negative correlation between average forest and road distances could partially explain the pattern of echinostome infection but not the *Alaria* sp. infection, given the lack of road effect for that species. Both aspects of forest distance as well as average road distance differed between

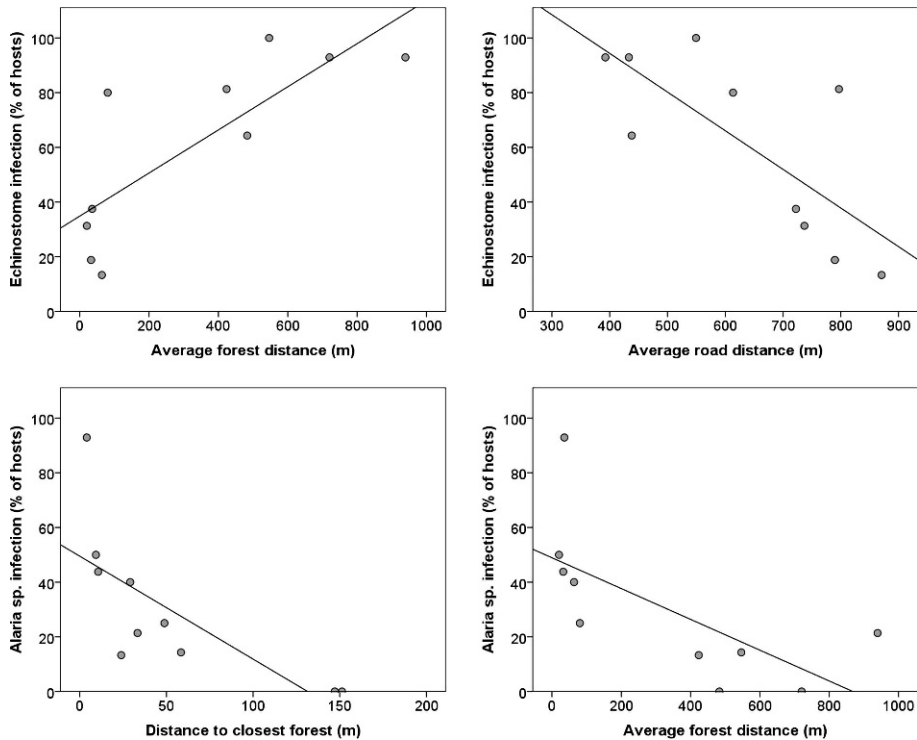


FIGURE 3. Scatter plots depicting statistically significant relationships among ponds means for grey tree frog *Hyla versicolor* tadpole infection and habitat variables. Lines of fit are only to illustrate the nature of the relationships.

the sampled soybean and nonagricultural ponds. In addition, the differences for both measures of forest between the two pond types were maintained for the larger set of 18 ponds, indicating general habitat differences between agricultural and nonagricultural sites. These findings suggest that the effects of agricultural activities on amphibian parasitism may be widespread if they can be driven by associated habitat perturbations regardless of contaminant effects. However, the relatively small sample sizes here (10 sampled ponds, 18 ponds overall for habitat measures) and in other studies of agriculture and amphibian parasitism (range of 5–18 ponds; e.g., King et al., 2007; Rohr et al., 2008b) highlight the need for larger-scale investigations to elucidate the importance of habitat/landscape.

Our findings are similar to those of other recent field studies regarding the

importance of examining wetland and landscape aspects in addition to agricultural contaminants for amphibian parasitism (King et al., 2007, 2008, 2010; McKenzie, 2007; Schotthoefer et al., 2011). A study focusing on corn agriculture in southern Ontario, Canada (using some of the same ponds as used in this study), found that the overall prevalence of trematode infection in grey tree frogs was higher in tadpoles from ponds adjacent to corn fields versus those from nonagricultural ponds (Koprivnikar et al., 2006). This result was attributed to the presence of the herbicide atrazine because habitat measures did not differ between the two pond types. In contrast, given the absence of glyphosate but the presence of general habitat differences between our two pond types in this study, the effects of soybean agriculture on amphibian trematode parasitism in the present study could

be attributed to habitat. We propose that the greater prevalence of echinostomes and lower *Alaria* sp. infection in tadpoles from ponds adjacent to soybean fields compared with nonagricultural ponds is likely attributable to differences in habitat between the two pond types but requires further examination. Notably, glyphosate may have present in our ponds at other times given the previously documented fluctuations in its detection from field sites (Struger et al., 2008) such that repeated sampling is optimal (King et al., 2008). In addition, we did not test for the presence of other pesticides that could have theoretically remained from applications in previous years or for the presence of glyphosate in tadpole tissues. Other factors, such as intermediate snail host abundance, should also be incorporated in similar future studies because trematode infection in these hosts is also influenced by landscape and agricultural activity (Koprivnikar et al., 2007).

Numerous reports now support the link between habitat suitability for various pathogens/hosts and patterns of infection. Habitat fragmentation, vegetation cover, and road presence have all been associated with effects on parasite prevalence or diversity (e.g., Urban, 2006; Vaz et al., 2007). Our findings are also consistent with studies examining the effects of habitat perturbations on communities in that those frequently show a high degree of specificity with respect to which species or groups are affected. Predators are particularly affected by alterations to the landscape, such as fragmentation and deforestation (e.g., Prieto-Benítez and Méndez, 2011), especially mammals, who often have specific habitat requirements and are negatively affected by reduced forest area and increased fragmentation (e.g., Brady et al., 2011). Habitat effects can also be dependent on whether natural enemies are considered to be generalists or specialists and these groups often exhibit contrasting responses to landscape alterations (e.g., Rand and Tschamtkke,

2007; Beasley et al., 2011). Because parasites can also be considered consumers in food web dynamics (Lafferty et al., 2008), we may expect specialist or generalist parasite species to exhibit different responses to habitat perturbations, similar to that observed for predators, which is supported by the current study and by others on amphibian parasitism.

Echinostome trematodes are widespread and common in amphibians (Johnson and McKenzie, 2008), with various echinostomes using a wide variety of both intermediate and definitive host species, including many birds and mammals for the latter (Olsen, 1974). This group of trematodes should then occur relatively frequently in perturbed habitats, compared with trematode species with greater host specificity. Our findings regarding echinostome occurrence correspond with King et al. (2007), who found only these trematodes in all sample localities and suggested that resulted from the ability of this generalist parasite group to persist in disrupted habitats. Although we found associations between average forest and road distances and echinostome prevalence, which probably does not reflect a preference for more open surroundings given the range of potential hosts (various mammals and birds). Rather, echinostomes can likely be maintained in a variety of habitats because it is highly probable that some of the animals using degraded sites are suitable final hosts. This is less likely for host-specialist species. Additionally, loss of habitat can lead to increased host crowding, as observed for wetland loss and migrating bird species, which can result in increased pathogen transmission (e.g., Blanchong et al., 2006). Such foci of trematode infection have been previously noted for areas where the host tends to aggregate at high densities (e.g., Hechinger and Lafferty, 2005) and likely occur as a result of host crowding stemming from habitat fragmentation (Nunn et al., 2003).

In comparison to the observed pattern of echinostome infection here, the occurrence

of a more–host-specific trematode group, *Alaria* sp., was rather restricted. The increased prevalence of *Alaria* sp. with decreasing distance to the closest forest patch and decreasing average forest distance is probably attributable to the importance of forest habitat for the small mammals serving as definitive hosts for this genus of trematodes (Olsen, 1974). This corresponds with previously reported findings regarding landscape influences on mammal-reliant trematode species (Koprivnikar et al., 2006; King et al., 2007). The relationship here between *Alaria* sp. and forest distance also likely reflects the importance of buffer zones surrounding aquatic habitats for the distribution of small mammals (Jones et al., 1988). Future examinations with larger sample sizes will be needed to firmly establish the differences between host-generalist and specialist parasites with respect to habitat perturbations.

Further studies are needed to verify our hypotheses that 1) agricultural effects on parasitism may be broad if habitat-mediated influences are common, and 2) generalist and specialist parasite species have differing sensitivities to habitat perturbations. These have significant implications for both wildlife disease and the use of parasites as bioindicators and gauges of ecosystem health. With respect to amphibian parasitism, an increase in the prevalence or intensity of trematode infections resulting from agricultural activities can have detrimental effects on individual hosts and, perhaps, populations; however, the identity of the parasite species is critical. Domination by generalist rather than specialist parasites can have important consequences regardless of whether overall prevalence of infection is affected or not.

Echinostome trematodes have intensity-dependent effects that are capable of causing edema and impairing osmoregulation and growth in young tadpoles to the extent that mortality resulting from infection can be similar to predation (Fried et al., 1997; Holland, 2010). Although its low occurrence

in this study precluded our ability to establish environmental correlations, *Ribeiroia ondatrae* is another generalist trematode of concern. This trematode species also causes high mortality in young tadpoles, as well as deformities in survivors, such that malformed frogs rarely survive to sexual maturity (Goodman and Johnson, 2011; Johnson et al., 2011). Consequently, reduced parasite diversity/richness in amphibians from perturbed sites does not mean that these individuals are in better health (King et al., 2007), particularly if infection with persisting parasite species interacts with other stressors common in agricultural landscapes to negatively affect hosts (e.g., Koprivnikar, 2010; Marcogliese and Pietrock, 2010). This is a relevant concern because many amphibians increasingly rely on wetlands in agricultural landscapes for breeding purposes as their natural habitats decline (Knutson et al., 1999, 2004).

The potentially important contribution of habitat-driven agricultural effects also has implications for the use of parasites as bioindicators and gauges of ecosystem health. Because many parasites, such as trematodes, rely on species across different trophic levels to complete their complex life cycles and can be directly sensitive to various environmental factors themselves, their presence and abundance can provide information regarding the activity, distribution, and condition of their hosts (Marcogliese and Cone, 1997; Sures, 2004; Marcogliese, 2005). The use of parasites to examine ecologic food webs and the effects of environmental perturbations has been termed *environmental parasitology* (Lafferty, 1997) and has been explored for other hosts, such as fish (e.g., MacKenzie et al., 1995), but parasites of amphibians have much potential in this capacity as well (King et al., 2008, 2010). However, this study and others (e.g., King et al., 2008; Chasar et al., 2009) have demonstrated that different parasite groups, and even species of the same group, do not necessarily show the same response to a particular environmental

perturbation. Although the presence of many parasite species may be more typical of a “healthy” ecosystem (Marcogliese, 2005; Hudson et al., 2006), dominance by generalist species may, in fact, indicate the opposite and pose a significant threat to host health, especially if both the intensity and prevalence of infection with some pathogenic species increase. Such context-dependent knowledge is necessary to better understand the effects of environmental perturbations on disease patterns and to more accurately assess risks to the health of amphibians and other wildlife of concern when developing conservation and management strategies.

ACKNOWLEDGMENTS

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and from the Brandon University Research Council to J.K., as well as an NSERC Undergraduate Student Research Assistantship (USRA) to J.C.R.

LITERATURE CITED

- BEASLEY, J., Z. H. OLSON, G. DHARMARAJAN, T. S. EAGAN II, AND O. E. RHODES, JR. 2011. Spatio-temporal variation in the demographic attributes of a generalist mesopredator. *Landscape Ecology* 26: 937–950.
- BLANCHONG, J. A., M. D. SAMUEL, AND G. MACK. 2006. Multispecies patterns of avian cholera mortality in Nebraska’s rainwater basin. *Journal of Wildlife Diseases* 42: 81–91.
- BRADLEY, C. A., AND S. ALTIZER. 2007. Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution* 22: 95–102.
- BRADY, M. J., C. A. MCALPINE, H. P. POSSINGHAM, C. J. MILLER, AND G. S. BAXTER. 2011. Matrix is important for mammals in landscapes with small amounts of native forest habitat. *Landscape Ecology* 26: 617–628.
- CHASAR, A., C. LOISEAU, G. VALKIUS-NAS, T. IEZHOVA, T. B. SMITH, AND R. N. SEHGAL. 2009. Prevalence and diversity patterns of avian blood parasites in degraded African rainforest habitats. *Molecular Ecology* 18: 4121–4133.
- CHRISTIN, M.-S., A. D. GENDRON, P. BROUSSEAU, L. MENARD, D. J. MARCOGLIESE, D. CYR, S. RUBY, AND M. FOURNIER. 2003. Effects of agricultural pesticides on the immune system of *Rana pipiens* and on its resistance to parasitic infection. *Environmental Toxicology and Chemistry* 22: 1127–1133.
- DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2003. Infectious disease and amphibian population declines. *Diversity and Distributions* 9: 141–150.
- FRIED, B., P. L. PANE, AND A. REDDY. 1997. Experimental infection of *Rana pipiens* tadpoles with *Echinostoma trivolvis* cercariae. *Parasitology Research* 83: 666–669.
- GOODMAN, B. A., AND P. T. J. JOHNSON. 2011. Disease and the extended phenotype: Parasites control host performance and survival through induced changes in body plan. *PLoS One* 6: e20193.
- HARVELL, C. D., C. E. MITCHELL, J. R. WARD, S. A. ALTIZER, A. P. DOBSON, R. S. OSTFELD, AND M. D. SAMUEL. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* 296: 2158–2162.
- HECHINGER, R. F., AND K. D. LAFFERTY. 2005. Host diversity begets parasite diversity: Bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society of London Series B* 272: 1059–1066.
- HOLLAND, M. P. 2010. Echinostome-induced mortality varies across amphibian species in the field. *Journal of Parasitology* 96: 51–855.
- HUDSON, P. J., A. P. DOBSON, AND D. NEWBORN. 1998. Prevention of population cycles by parasite removal. *Science* 282: 2256–2258.
- , ———, AND K. D. LAFFERTY. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution* 21: 381–385.
- JOHNSON, P. T. J., AND J. M. CHASE. 2004. Parasites in the food web: linking amphibian malformations and aquatic eutrophication. *Ecology Letters* 7: 521–526.
- , AND V. J. MCKENZIE. 2008. Effects of environmental change on Helminth infections in Amphibians: Exploring the emergence of *Ribeiroia* and *Echinostoma* infections in North America. In *The biology of echinostomes*, B. Fried and R. Toledo (eds.). Springer, New York, New York, pp. 250–280.
- , AND S. H. PAULL. 2011. The ecology and emergence of diseases in fresh waters. *Freshwater Biology* 56: 638–657.
- , E. KELLERMANN, AND J. BOWERMAN. 2011. Critical windows of disease risk: Amphibian pathology driven by developmental changes in host resistance and tolerance. *Functional Ecology* 25: 726–734.
- JONES, J. J., J. P. LORTIE, AND U. D. PIERCE. 1988. The identification and management of significant fish and wildlife resources in southern coastal Maine. Maine Department of Inland Fisheries and Wildlife, Augusta, Maine, 14 pp.
- KELLY, D. W., R. POULIN, D. M. TOMPKINS, AND C. R. TOWNSEND. 2010. Synergistic effects of glyphosate formulation and parasite infection on fish malformations and survival. *Journal of Applied Ecology* 47: 498–504.

- KIESECKER, J. M. 2002. Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? *Proceedings of the National Academy of Sciences U. S. A.* 99: 9900–9904.
- KILLILEA, M. E., A. SWEI, R. S. LANE, C. J. BRIGGS, AND R. S. OSTFELD. 2008. Spatial dynamics of Lyme disease: a review. *Ecohealth* 5: 167–195.
- KING, K. C., J. D. McLAUGHLIN, A. D. GENDRON, B. D. PAUL, I. GIROUX, B. RONDEAU, M. BOILY, P. JUNEAU, AND D. J. MARCOGLIESE. 2007. Impacts of agriculture on the parasite communities of northern leopard frogs (*Rana pipiens*) in southern Quebec, Canada. *Parasitology* 134: 2063–2080.
- , A. D. GENDRON, J. D. McLAUGHLIN, I. GIROUX, P. BROUSSEAU, D. CYR, S. M. RUBY, M. FOURNIER, AND D. J. MARCOGLIESE. 2008. Short-term seasonal changes in parasite community structure in northern leopard froglets (*Rana pipiens*) inhabiting agricultural wetlands. *Journal of Parasitology* 94: 13–22.
- , J. D. McLAUGHLIN, M. BOILY, AND D. J. MARCOGLIESE. 2010. Effects of agricultural landscape and pesticides on parasitism in native bullfrogs. *Biological Conservation* 143: 302–310.
- KNUTSON, M. G., J. R. SAUER, D. A. OLSEN, M. J. MOSSMAN, L. M. HEMESATH, AND M. J. LANNOO. 1999. Effects of landscape composition and wetland fragmentation on frog and toad abundance and species richness in Iowa and Wisconsin, USA. *Conservation Biology* 13: 1437–1446.
- , W. B. RICHARDSON, D. M. REINEKE, B. R. GRAY, J. R. PARMELEE, AND S. E. WEICK. 2004. Agricultural ponds support amphibian populations. *Ecological Applications* 14: 669–684.
- KOPRIVNIKAR, J. 2010. Interactions of environmental stressors impact survival and development of parasitized larval amphibians. *Ecological Applications* 20: 2263–2272.
- , M. R. FORBES, AND R. L. BAKER. 2006. Environmental factors influencing trematode prevalence in grey tree frog (*Hyla versicolor*) tadpoles in southern Ontario. *Journal of Parasitology* 92: 997–1001.
- , R. L. BAKER, AND M. R. FORBES. 2007. Environmental factors influencing community composition of gastropods and their trematode parasites in southern Ontario. *Journal of Parasitology* 93: 992–998.
- LAFFERTY, K. D. 1997. Environmental parasitology: What can parasites tell us about human impacts on the environment? *Parasitology Today* 13: 251–255.
- , AND R. D. HOLT. 2003. How should environmental stress affect the population dynamics of disease? *Ecology Letters* 6: 654–664.
- LAFFERTY, K. D., S. ALLESINA, M. ARIM, C. J. BRIGGS, G. DE LEO, A. P. DOBSON, J. A. DUNNE, P. T. J. JOHNSON, A. M. KURIS, D. J. MARCOGLIESE, N. D. MARTINEZ, J. MEMMOTT, P. A. MARQUET, J. D. McLAUGHLIN, E. A. MORDECAI, M. PASCUAL, R. POULIN, AND D. W. THIELTGES. 2008. Parasites in food webs: The ultimate missing links. *Ecology Letters* 11: 533–546.
- LEHRER, E. W., S. L. FREDERBAUGH, R. L. SCHOOLEY, AND N. E. MATEUS-PINILLA. 2010. Prevalence of antibodies to *Toxoplasma gondii* in woodchucks across an urban-rural gradient. *Journal of Wildlife Diseases* 46: 977–980.
- MACKENZIE, K., H. H. WILLIAMS, B. WILLIAMS, A. H. McVICAR, AND R. SIDDALL. 1995. Parasites as indicators of water quality and the potential use of helminth transmission in marine pollution studies. *Advances in Parasitology* 35: 85–144.
- MARCOGLIESE, D. J. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? *International Journal for Parasitology* 35: 705–716.
- , AND D. K. CONE. 1997. Parasite communities as indicators of ecosystem stress. *Parasitología* 39: 227–232.
- , AND M. PIETROCK. 2010. Combined effects of parasites and contaminants on animal health: Parasites do matter. *Trends in Parasitology* 27: 123–130.
- MCKENZIE, V. J. 2007. Human land use and patterns of parasitism in tropical amphibian hosts. *Biological Conservation* 137: 102–116.
- MOLYNEUX, D. H. 2003. Common themes in changing vector-borne disease scenarios. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 97: 129–132.
- NUNN, C. L., S. M. ALTIZER, K. E. JONES, AND W. SECHREST. 2003. Comparative tests of parasite species richness in primates. *American Naturalist* 162: 598–614.
- OLSEN, O. W. 1974. *Animal parasites: Their life cycles and ecology*. 3rd Edition. University Park Press, Baltimore, Maryland, 562 pp.
- PATZ, J. A., T. K. GRACZYK, N. GELLER, AND A. Y. VITTOR. 2000. Effects of environmental change on emerging parasitic diseases. *International Journal for Parasitology* 30: 1395–1405.
- PAWLAK, J. A., J. J. KELLS, M. BARRETT, AND W. F. MEGGITT. 1987. Effect of atrazine residue on soybean growth under three tillage systems and various herbicides. *Weed Technology* 1: 140–144.
- PRIETO-BENÍTEZ, S., AND M. MÉNDEZ. 2011. Effects of land management on the abundance and richness of spiders (Araneae): A meta-analysis. *Biological Conservation* 144: 683–691.
- RALIN, D. B. 1968. Ecological and reproductive differentiation in the cryptic species of the *Hyla versicolor* complex (Hylidae). *Southwestern Naturalist* 13: 283–299.

- RAND, T. A., AND T. TSCHARNTKE. 2007. Contrasting effects of natural habitat loss on generalist and specialist aphid natural enemies. *Oikos* 116: 1353–1362.
- ROHR, J. R., AND K. A. MCCOY. 2010. A quantitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. *Environmental Health Perspectives* 118: 20–32.
- , T. R. RAFFEL, S. K. SESSIONS, AND P. J. HUDSON. 2008a. Understanding the net effects of pesticides on amphibian trematode infections. *Ecological Applications* 18: 1743–1753.
- , A. M. SCHOTTHOEFER, T. R. RAFFEL, H. J. CARRICK, N. HALSTEAD, J. T. HOVERMAN, C. M. JOHNSON, L. B. JOHNSON, C. LIESKE, M. D. PIWONI, P. K. SCHOFF, AND V. R. BEASLEY. 2008b. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* 455: 1235–1239.
- SHELL, S. C. 1970. How to know the trematodes. W. C. BROWN Company, Dubuque, Iowa, 355 pp.
- . 1985. Handbook of trematodes of North America, north of Mexico. University Press of Idaho, Moscow, Idaho, 263 pp.
- SCHOTTHOEFER, A. M., J. R. ROHR, R. A. COLE, A. V. KOEHLER, C. M. JOHNSON, L. B. JOHNSON, AND V. R. BEASLEY. 2011. Effects of wetland and landscape variables on parasite communities of *Rana pipiens*: links to anthropogenic changes. *Ecological Applications* 21: 1257–1271.
- SEHGAL, R. N. M. 2010. Deforestation and avian infectious diseases. *Journal of Experimental Biology* 213: 955–960.
- STRUGER, J., D. THOMPSON, B. STAZNIK, P. MARTIN, T. MCDANIEL, AND C. MARVIN. 2008. Occurrence of glyphosate in surface waters of southern Ontario. *Bulletin of Environmental Contamination and Toxicology* 80: 378–384.
- SURES, B. 2004. Environmental parasitology: Relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology* 20: 170–177.
- SZUROCZKI, D., AND J. M. L. RICHARDSON. 2009. The role of trematode parasites in larval anuran communities: an aquatic ecologist's guide. *Oecologia* 161: 371–385.
- URBAN, M. C. 2006. Road facilitation of trematode infections in snails of northern Alaska. *Conservation Biology* 20: 1143–1149.
- VAZ, V. C., P. S. D'ANDREA, AND A. M. JANSEN. 2007. Effects of habitat fragmentation on wild mammal infection by *Trypanosoma cruzi*. *Parasitology* 134: 1785–1793.

Submitted for publication 2 September 2011.

Accepted 2 April 2012.