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Nematode parasites and leukocyte profiles of Northern Leopard Frogs, *Rana pipiens*: location, location, location

Dave Shutler, Andrée D. Gendron, Myriam Rondeau, and David J. Marcogliese

Abstract: Globally, amphibians face a variety of anthropogenic stresses that include exposure to contaminants such as agricultural pesticides. Pesticides may negatively affect amphibian immune systems, concomitantly increasing susceptibility to parasitism. We quantified nematodes and evaluated leukocyte profiles of Northern Leopard Frogs (*Rana pipiens* Schreber, 1782) collected from five wetlands in southwestern Quebec, Canada, that spanned a gradient of pesticide exposure. Three taxa of nematode parasites (*Rhabdias ranae* Walton, 1929, genus *Oswaldocruzia* Travassos, 1917, and genus *Strongyloides* Grassi, 1879) were sufficiently numerous for detailed evaluation. When all frogs were pooled, frog size was negatively correlated with nematode species richness, abundances of each of the three nematode species, and densities of three different leukocytes. When all frogs were pooled, there was strong evidence of both negative and positive associations between pairs of parasite species. However, none of the previous relationships was significant within wetlands. Our results reveal strong spatial organization of amphibian–parasite communities and illustrate the importance of controlling for sampling locale in evaluating host–parasite associations. Finally, although several response variables varied significantly among wetlands, causes of this variation did not appear to be related to variation in nematode parasitism or pesticide exposure.

Key words: leucocytes, nematodes, Northern Leopard Frog, *Oswaldocruzia* sp., pesticides, *Rana pipiens*, *Rhabdias ranae*, *Strongyloides* sp.

Résumé : À l'échelle planétaire, les amphibiens sont confrontés à divers stress d'origine humaine incluant l'exposition à des contaminants tels que des pesticides agricoles. Ces derniers peuvent avoir une incidence négative sur les systèmes immunitaires des amphibiens, les rendant du coup plus sensibles au parasitisme. Nous avons quantifié les nématodes et évalué les profils de leucocytes de grenouilles léopards (*Rana pipiens* Schreber, 1782) prélevées dans cinq milieux humides du sud-ouest du Québec (Canada) étalés le long d'un gradient d'exposition aux pesticides. Trois taxons de nématodes parasites (*Rhabdias ranae* Walton, 1929, le genre *Oswaldocruzia* Travassos, 1917 et le genre *Strongyloides* Grassi, 1879) étaient suffisamment nombreux pour permettre une évaluation détaillée. Quand toutes les grenouilles étaient regroupées, leur taille était négativement corrélée à la richesse spécifique des nématodes, à l'abondance de chacune des trois espèces de nématodes et aux densités de trois leucocytes distincts. Quand toutes les grenouilles étaient regroupées, il y avait de fortes indications d'associations tant négatives que positives entre paires d'espèces de parasites. Cependant, aucune de ces relations n'était significative à l'échelle du milieu humide individuel. Nos résultats révèlent une forte organisation spatiale des communautés de parasites d'amphibiens et illustrent l'importance de tenir compte du lieu d'échantillonnage dans l'évaluation des associations hôte–parasite. Enfin, si plusieurs variables de réaction différaient significativement d'un milieu humide à l'autre, les causes de ces différences ne semblaient pas reliées à des différences sur le plan du parasitisme par les nématodes ou de l'exposition aux pesticides. [Traduit par la Rédaction]

Mots-clés : leucocytes, nématodes, grenouille léopard, *Oswaldocruzia* sp., pesticides, *Rana pipiens*, *Rhabdias ranae*, *Strongyloides* sp.

Introduction

Many populations of amphibians are now relegated to wetlands in fragmented agricultural landscapes (Knutson et al. 2004). In these wetlands, amphibians are often exposed to runoff that contains contaminants such as pesticides (Cabagna et al. 2005; King et al. 2010). Pesticides may weaken immune systems (Hoole 1997; Christin et al. 2003, 2004; Gilbertson et al. 2003), increasing amphibian vulnerability to parasites and increasing both prevalences and intensities (Kiesecker 2002; Forson and Storfer 2006; Rohr et al. 2008). However, parasites themselves may be negatively affected by pesticides (Pietrock and Marcogliese 2003). In addition, landscape fragmentation may interrupt parasite transmission opportunities, leading to lower prevalences (e.g., Taylor and Merriam 1996; King et al. 2008, 2010). Empirical data are necessary to evaluate which of these alternatives operates in a particular system.

In some landscapes, wetlands are far more patchily distributed than are terrestrial habitats, which can limit movement of wetland-restricted hosts and (or) parasites. This may produce isolated host and parasite populations that experience different selection pressures. For example, parasite communities are often significantly reduced and differentiated on islands compared with the mainland, likely in part because of restricted movements of both host and parasite taxa (Apanius et al. 2000; Fallon et al. 2003; Beadell et al. 2007). When the predominant habitat is terrestrial, wetlands may become functionally similar to islands with parallels in how their resident species assemble. Thus, amphibians from spatially separated wetlands may have very different parasite communities.

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Host body size is also hypothesized to affect prevalence and intensity of parasites (Poulin 2007). For example, large hosts may be exposed more often to parasites (e.g., because of increased food intake). In contrast, parasites may stunt growth or reduce survival so that only individuals with few parasites grow to a large size. Another possibility is that larger hosts may be healthy individuals with immune systems better able to prevent parasites from establishing or at purging those already established (Wilson et al. 2001; Poulin 2007). Immune systems are one of the last remaining options that hosts have to mitigate consequences of parasites that enter their bodies. Strength of immune response (immunocompetence) can be influenced by various aspects of host health such as exposure to pesticides (Carey et al. 1999, 2003; Voccia et al. 1999) and parasites (particularly if there are parasite synergisms or antagonisms). Immunocompetence may differ for different parasites (e.g., because of variation in virulence or variation in ability to evade detection by immune systems). Leukocyte profiles are metrics of immune investment that are widely and effectively used in ecological studies (Vleck et al. 2000; Shutler et al. 2004; Davis et al. 2008; Budischak et al. 2012). Nonetheless, although leukocyte profiles may reflect pre-emptive investment against parasites, they may also reflect investment against current infections or responses to a variety of other environmental variables (e.g., temperature; Raffel et al. 2006), so that interpretation of these profiles needs to be made cautiously (Norris and Evans 2000).

Shutler and Marcogliese (2011) reported significant differences in leukocyte profiles of Northern Leopard Frogs (*Rana pipiens* Schreber, 1782; *Lithobates pipiens* (Schreber, 1782) according to some authorities (Frost et al. 2006) but not others (Stuart 2008; Pauly et al. 2009)) among wetlands in southern Quebec, Canada. When reference sites were pooled and compared with pooled herbicide-exposed sites, there was some suggestion that frogs in the latter sites had more evidence of stress (higher neutrophil:lymphocyte ratios). There was also some evidence that frogs infected with blood parasite species of the genus *Hepatozoon* Miller, 1908 (Protozoa: Apicomplexa) had fewer eosinophils and had higher neutrophil:lymphocyte ratios, although most (86%) infected frogs came from a single wetland. Earlier studies in the same wetlands demonstrated that three parasitic nematodes (*Rhabdias ranae* Walton, 1929, genus *Oswaldocruzia* Travassos, 1917, genus *Strongyloides* Grassi, 1879), all with direct life cycles, were more prevalent and abundant in Northern Leopard Frogs from wetlands in agricultural landscapes compared with those in reference wetlands (King et al. 2008). King et al. (2008) suggested that pesticide exposure negatively affected frogs' immune responses, leading to increased nematode infections. Accordingly, Gendron et al. (2003) found that more *R. ranae* established, and established faster, in lungs of frogs exposed versus not exposed to pesticides. Here, we repeated the sampling regime outlined in King et al. (2008) to further test that hypothesis. In addition, given that exposure to pesticides and the nematode *R. ranae* appeared to cause a reduction in lymphocyte numbers in the spleen (Christin et al. 2003, 2004), we tested whether nematodes in agricultural wetlands are a potential explanation for differences in leukocyte profiles observed in Shutler and Marcogliese (2011). We also tested whether frog sex and metrics of frog size were associated with variation in nematode parasitism, and for evidence of positive or negative association between pairs of nematode species. We first analyzed relationships pooling frogs from all wetlands, and to validate generality of those relationships, reanalysed data within wetlands.

Materials and methods

Study area

Additional details on our study area and methods are provided elsewhere (King et al. 2007, 2008; Shutler and Marcogliese 2011). Briefly, we studied Northern Leopard Frogs in five wetlands (Fig. 1) that were evaluated for several pesticides at Centre d'expertise en analyses environnemental du Québec; two pesticides that merited the most attention were the herbicides atrazine and metolachlor because of their extensive use in southern Quebec and because of their documented effects on amphibians (Christin et al. 2003; Rohr and McCoy 2010). Water was collected for pesticide analysis weekly between 23 May and 25 July 2006 at Rivière Chibouet (hereafter Chibouet; draining extensive agricultural landscapes) and Ruisseau Fairbanks (Fairbanks; adjacent to farmland) and biweekly between 23 May and 18 July 2006 at Étang John Sauro (Sauro; a conservation area managed by Ducks Unlimited), Parc le Rocher (Rocher; a managed wetland situated in a municipal park), and Île de la Commune (Commune; adjacent to farmland in a provincial park where atrazine use is now forbidden). Atrazine and metolachlor are normally applied in June in southern Quebec when Northern Leopard Frog tadpoles are undergoing larval development, a stage that may be sensitive to contaminants and that may affect later immunological and endocrinological functions (Boily et al. 2005; Carey and Bryant 1995; CCME 2010; Lehman and Williams 2010; Rohr and McCoy 2010). Mean, minimum, and maximum distances between any pair of wetlands were 57.0, 11.6, and 95.6 km, respectively, making autocorrelation unlikely because these distances exceed known Northern Leopard Frog movements (Schotthoefer et al. 2011).

Collection and identification

Collection procedures adhered to guidelines of Canadian Council on Animal Care (2003). Following methods outlined in King et al. (2008), juvenile frogs approximately 7–8 weeks after metamorphosis were collected from wetlands between 5 and 11 September 2006 by hand or dip net. Frog snout–vent length (SVL) was measured with Vernier calipers to the nearest 0.01 mm and mass was measured with an electronic balance to the nearest 0.01 g. Frogs at this age were unlikely to have dispersed so it is likely that they were exposed to local pesticides within each wetland (King et al. 2007, 2008).

When captured, each frog heart was punctured with a sterile 27-gauge needle and approximately 20–40 mL of blood were collected in a heparinized capillary tube before being smeared on a microscope slide and fixed in alcohol (Bennett 1970). Frogs were subsequently killed in an overdose of buffered 0.8% tricaine methansulfonate (MS222; Boreal Science, St. Catharines, Ontario, Canada) and bodies were frozen.

Sex was determined from examination of the gonads. Lungs, digestive tracts, and body cavities of each frog were examined for parasitic nematodes. Cysts containing larval nematodes were either torn open mechanically or chemically dissolved by exposing them briefly to a diluted hypochlorite solution (described in Gendron et al. 2012). Parasite specimens were cleaned, enumerated, and preserved in an aqueous solution of 70% ethanol and 5% glycerol. Worms were later cleared by progressive evaporation of ethanol in the storage solution and transferred to pure glycerol before being mounted on slides. Identifications were based on published descriptions (Hedrick 1935; Baker 1978; Vanderburgh and Anderson 1987; Speare 1989; Ben Slimane and Durrette-Desset 1997; McAlpine and Burt 1998; Kuzmin et al. 2003). Voucher specimens were deposited in the Royal Ontario Museum (Toronto, Ontario, Canada): *Rhabdias ranae* (ROMIZ F331 and F332); species of the genera *Cosmocercoides* Wilkie, 1930 (ROMIZ F335), *Oswaldocruzia* (ROMIZ F333 and F334), *Spiroxys* Schneider, 1866 (ROMIZ F337), and *Strongyloides* (ROMIZ F336).

Blood smears were collected from 82 frogs, fixed and stained with Protocol Hema 3 (Biochemical Sciences Inc., Swedesboro, New Jersey, USA), and examined, blind to origin, at 1000× magnification. For each smear, we identified types of the first 200 leukocytes encountered, calculated percentage of each leukocyte type, quantified number of erythrocytes per field, and quantified number of microscope fields that we scanned to find 200 leukocytes. Each leucocyte plays a different role in immune function

Fig. 1. Study area in southern Quebec indicating locations of five wetlands from which Northern Leopard Frogs (*Rana pipiens*) were sampled (1 = Sauro; 2 = Rocher; 3 = Commune; 4 = Fairbanks; 5 = Chibouet).

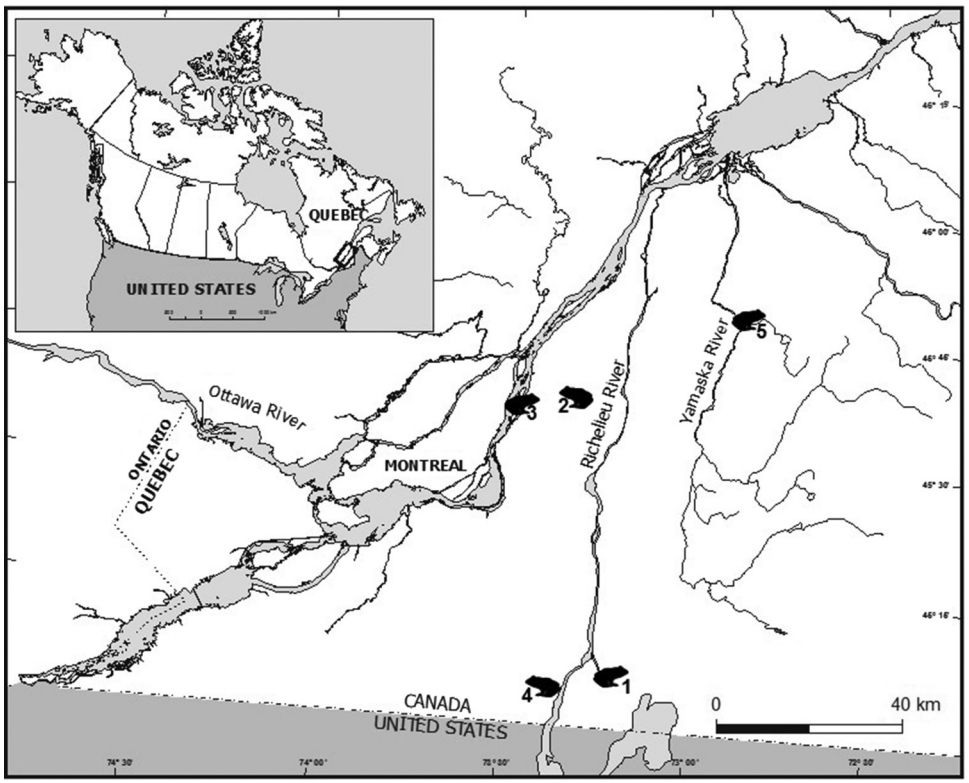


Table 1. Minimum (min.), maximum (max.), and mean measurements (μg/L) of atrazine and its derivatives and metolachlor between late May and July at study sites in 2006.

	Site (no. of water samples; pesticide ranking)														
	Sauro (5; 1)			Rocher (5; 2)			Commune (4; 3)			Fairbanks (9; 4)			Chibouet (9; 5)		
Pesticide	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Atrazine	<DL	<DL	<DL	<DL	0.020	0.008	<DL	0.080	0.058	0.040	1.000	0.170	0.070	4.400	1.089
Deethylatrazine	<DL	<DL	<DL	<DL	<DL	<DL	<DL	0.060	0.043	<DL	0.130	0.021	<DL	0.320	0.151
Deisopropylatrazine	<DL	0.040	0.008	<DL	0.030	0.006	<DL	0.040	0.010	<DL	0.040	0.010	<DL	0.170	0.047
Metolachlor	<DL	0.010	0.004	<DL	0.010	0.004	0.010	0.610	0.165	<DL	0.530	0.084	0.040	7.100	1.219

Note: "<DL" indicates readings that were below detectable levels.

(e.g., neutrophils are phagocytic) and so leucocyte profiles can provide clues about the health of a host (Davis et al. 2008). We computed neutrophil:lymphocyte ratios as a potential measure of response to parasite and pesticide stressors (Gross and Siegel 1983; Forbes et al. 2006; Davis et al. 2008). Density of each leukocyte was computed as the proportion of leukocytes per 1000 erythrocytes. Ten randomly chosen slides were re-examined to confirm repeatability of data (Shutler and Marcogliese 2011). Because they made up only a small proportion of leukocytes, basophil and monocyte counts (terminology follows Wright 2001) were not statistically repeatable ($P > 0.05$; Shutler and Marcogliese 2011), so we did not analyse relationships of other variables to these leukocytes.

Statistical analyses

Statistical analyses were performed in SAS version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). Because pesticide concentrations fluctuate annually, we ranked the five wetlands based on previous pesticide analyses (King et al. 2007; low ranks indicate low pesticide readings). Concentrations of atrazine and its derivatives and metolachlor were lowest in Sauro, followed by Rocher, Commune, and Fairbanks, and highest in Chibouet (Table 1). For most analyses involving parasite or leukocyte data, we used non-parametric statistics because those data were not normally distributed (Shapiro–Wilk tests, $P < 0.05$). We compared species richness and parasite abundances of females and males using Kruskal–Wallis tests. We used Spearman rank correlations (r_S) to test whether body size of frogs was associated with nematode species richness and abundances of each nematode (terminology follows Bush et al. 1997). We tested with ANOVAs whether frog body size differed among wetlands. We tested with contingency table analyses (χ^2 tests where expected values of all cells were >5, Fisher's exact tests where this was not the case) for associations among nematodes. We tested for correlations between leukocytes and frog body size, leukocytes and nematode species richness, and between leukocytes and abundance of each common species of nematode. We also tested whether nematode intensities were related to wetland pesticide rankings. We next repeated each of these tests within wetlands to assess generality of findings.

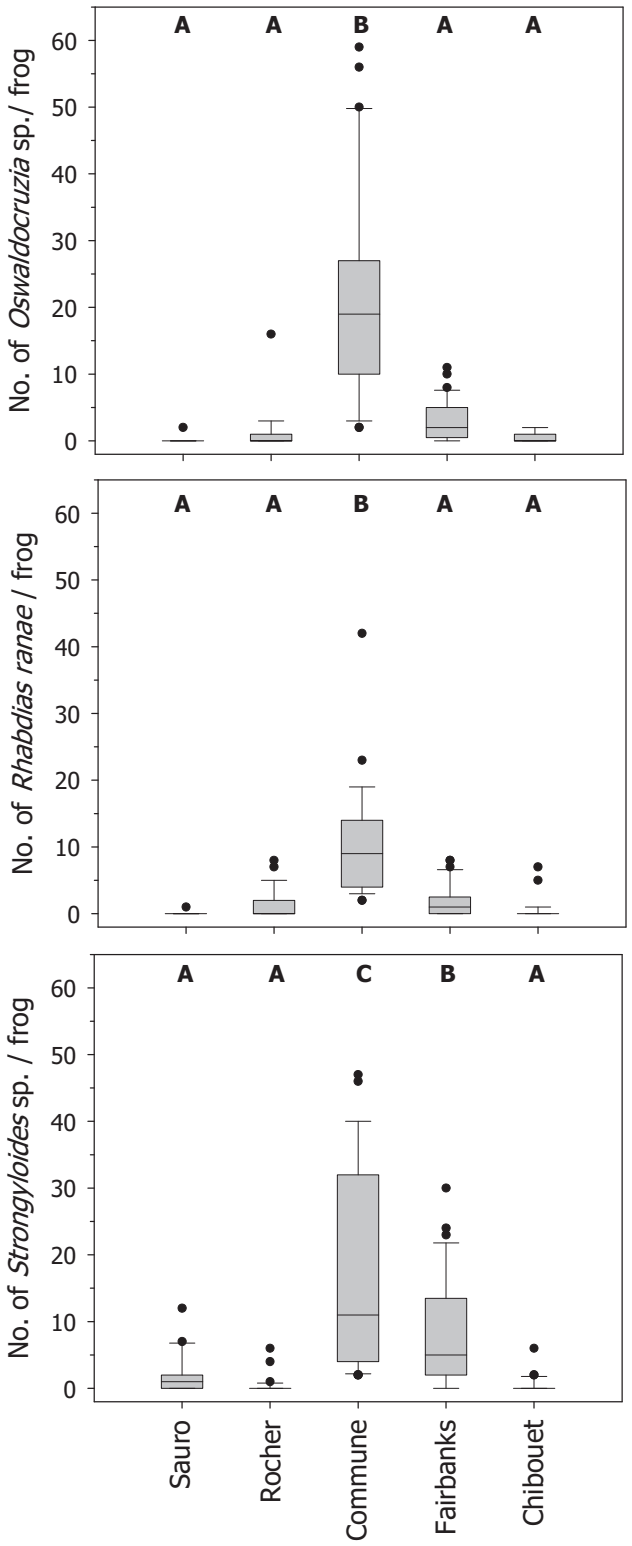
Results

Overall patterns

Parasites belonging to five nematode genera were found among 146 frogs: *Cosmocercoides*, *Oswaldocruzia*, *Rhabdias*, *Spiroxys*, and

Strongyloides. Of these frogs, 26 (18%) had no nematodes, 48 (33%) had one species, 24 (16%) had two species, 45 (31%) had three species, and 3 (2%) had four species. *Cosmocercoides* sp. was found in only 5 (3%) frogs and *Spiroxys* sp. was found in only 3 (2%) frogs, so we included data from these taxa only to compute nematode species richness. Abundance of each nematode was highest at Commune, but was not significantly different among other sites with the exception of higher abundance of *Strongyloides* sp. at Fairbanks relative to the other three wetlands (Fig. 2).

Fig. 2. Abundances of three common nematodes in Northern Leopard Frogs (*Rana pipiens*) differed among five different wetlands in southern Quebec (all $\chi^2_{[4]} \geq 82.8$, all $P < 0.0001$; wetlands sharing letters had statistically similar abundances for that nematode). Boxes encompass the middle 50% of data; horizontal line within boxes denote medians; vertical lines denote range of data excluding outliers that are designated with solid circles. Wetlands are arranged left to right in order of increasing pesticide concentrations.



All frogs pooled

Female and male frogs had similar nematode loads (all Kruskal–Wallis tests, $\chi^2_{[1]} < 3.4$, all $P > 0.07$). There was strong evidence of lower nematode species richness and lower abundances of each of the three common nematodes in larger frogs (Table 2; Fig. 3 illustrates relationships between total abundance and SVL and species richness and SVL; all eight analyses of size (mass and SVL) versus abundances and species richness had $r_S \leq -0.41$, all Ps < 0.0001). In analyses of associations between pairs of nematodes, frogs were more likely than expected by chance to have neither or both species in all cases (Table 3). We had leukocyte data for 82 frogs; 15 out of 28 correlations between leukocyte and nematode parameters were statistically significant (Table 4), whereas only 1.4 significant results were expected by chance. Lower proportions of neutrophils were found in frogs with more nematodes, whereas the opposite relationships were observed for densities of neutrophils. Higher proportions and densities of eosinophils and higher densities of lymphocytes were associated with more nematodes (Table 4). Finally, there were significant negative correlations between density (but not percentage) of each of the three leukocytes and frog size (both SVL and mass; all $r_S \leq -0.24$, all $P \leq 0.05$).

There were no significant relationships between wetland pesticide ranking and abundances of the three common nematodes (Fig. 2; all $|r_S| \leq 0.06$, all $P > 0.45$). However, pesticide ranking was significantly correlated with four of our seven leukocyte metrics (r_S with percent neutrophils = –0.28, $P = 0.01$; r_S with density of neutrophils = –0.08, $P = 0.47$; r_S with percent eosinophils = –0.14, $P = 0.22$; r_S with density of eosinophils = –0.03, $P = 0.77$; r_S with percent lymphocytes = 0.32, $P = 0.003$; r_S with density of lymphocytes = 0.33, $P = 0.002$; and r_S with neutrophil:lymphocyte ratio = –0.31, $P = 0.004$).

Patterns within wetlands

To validate the generality of the preceding patterns, we reanalysed data within wetlands. Nematode intensities of females differed from males in 4 of 15 analyses (5 wetlands × 3 nematode genera; males had more *Oswaldocruzia* sp. in Commune, females had more *R. ranae* in Fairbanks, and females had more *Strongyloides* sp. in Fairbanks, whereas males had more *Strongyloides* sp. in Chibouet; statistics in Table 5). Frog SVL and mass differed significantly among wetlands (Table 2), but these differences were not explained by ranked pesticide exposure (r_S for SVL = –0.09, $P = 0.25$; r_S for mass = –0.06, $P = 0.47$). Significant correlations in the pooled sample appeared to be driven mostly by frogs in Commune that were smaller and had higher parasite abundances. In contrast to the negative among-wetland patterns (e.g., Fig. 3), there was no significant consistent direction of associations between nematode species richness and abundances and frog size (23 out of 40 relationships were positive; binomial test, $P = 0.34$). However, the only two significant correlations within wetlands were that there was higher nematode richness ($r_S = 0.43$, $P = 0.02$) and lower abundance of *Strongyloides* sp. ($r_S = -0.36$, $P = 0.05$) in larger frogs from Commune (remaining $|r_S| \leq 0.34$, $P \geq 0.07$) (2 size measures × 4 parasite measures × 5 wetlands = 40 possible × 0.05 = 2 significant expected by chance).

Within wetlands, there was no significant association between any pairs of parasite taxa (Table 6). The reasons pooled data suggested nonrandom nematode associations are that frogs in Sauro

Table 2. Mean and standard deviations (SD) of size measures of Northern Leopard Frogs (*Rana pipiens*) among wetlands and relationship with nematode richness (Spearman rank correlations; r_S) within wetlands.

Wetland	*N*	Mean snout–vent length (SVL; mm)	SD	Group	r_S between SVL and richness	Mean mass (g)	SD	Group	r_S between mass and richness
Sauro	20	46.5	4.5	A	0.15	8.4	3.0	B	0.10
Rocher	30	48.4	3.5	A	0.02	11.4	2.5	A	0.31
Commune	31	34.2	3.9	B	**0.43**	3.7	1.2	C	0.32
Fairbanks	33	36.8	3.5	B	–0.17	4.9	1.3	C	–0.04
Chibouet	31	48.1	4.1	A	0.27	10.6	2.9	A	0.33

Note: Wetlands with lower pesticide concentrations appear first. Morphological measures were significantly different among wetlands (ANOVA; SVL: $R^2 = 0.72$, $F_{[4,140]} = 91.9$, $P < 0.0001$; mass: $R^2 = 0.67$, $F_{[4,140]} = 70.5$, $P < 0.0001$). Wetlands sharing a letter had frogs of similar morphology (Tukey's Studentized range test). One significant ($P = 0.02$) correlation between frog size and nematode richness is in boldface type.

Fig. 3. Relationship between total abundance of nematodes and nematode species richness of Northern Leopard Frogs (*Rana pipiens*) in southern Quebec relative to host snout–vent length. The relationship with total abundance was best described by a quadratic regression ($R^2 = 0.39$; overall model: $F_{[2,142]} = 46.0$, $P < 0.0001$; linear term: $F_{[1,142]} = 11.0$, $P = 0.001$; quadratic term: $F_{[1,142]} = 6.9$, $P = 0.01$).

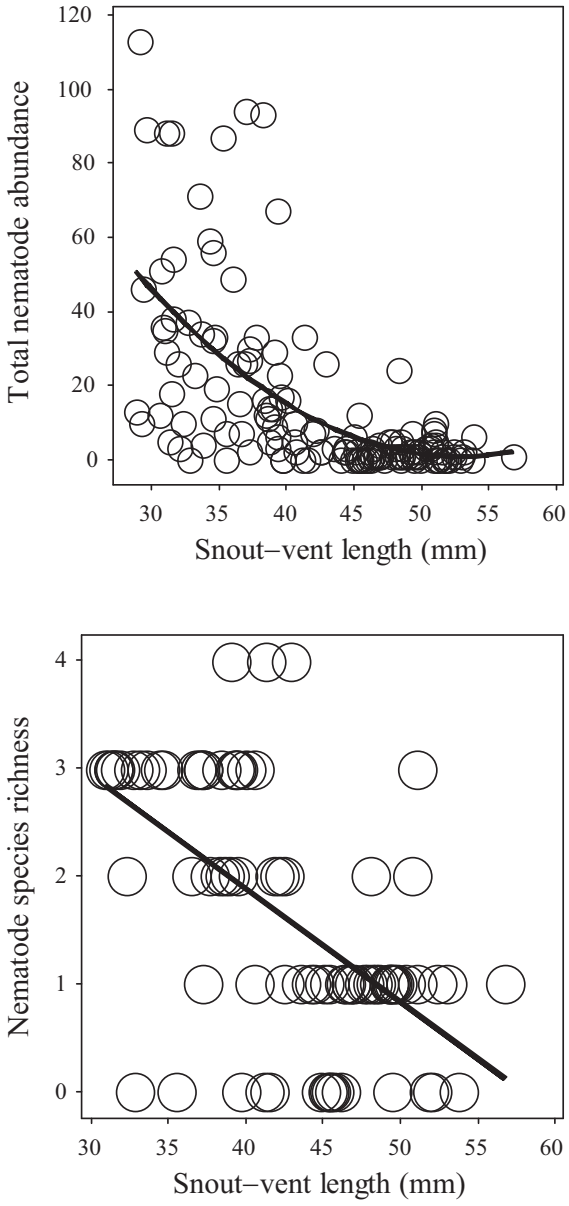


Table 3. Rates of coinfection with the three common genera for the pooled sample of Northern Leopard Frogs (*Rana pipiens*).

	Rhabdias ranae	
Oswaldocruzia sp.	Absent (expected)	Present (expected)
Absent	72 (54.2)	14 (31.8)
Present	20 (37.8)	40 (22.2)
	Strongyloides sp.	
Oswaldocruzia sp.	Absent (expected)	Present (expected)
Absent	62 (44.8)	24 (41.2)
Present	14 (31.2)	46 (28.8)
	Strongyloides sp.	
Rhabdias ranae	Absent (expected)	Present (expected)
Absent	62 (47.9)	30 (44.1)
Present	14 (28.1)	40 (25.9)

Note: All patterns are highly significant (all $\chi^2_{[1]} > 9.2$, all $P < 0.002$).

had no *R. ranae*, whereas all frogs at Commune were infected with all three of the common nematodes (Table 6).

There were no significant correlations between blood parameters and parasitism within Sauro ($N = 18$ frogs), Fairbanks ($N = 17$ frogs), or Chibouet ($N = 13$ frogs). At Rocher ($N = 16$ frogs), percentage and density of neutrophils were positively, percentage and density of lymphocytes were negatively, and neutrophil:lymphocyte ratios were positively correlated with abundance of *Oswaldocruzia* sp. (all $|r_S| \geq 0.51$, all $P \leq 0.04$). In addition, percentage of neutrophils was negatively associated with abundance of *Strongyloides* sp. and density of neutrophils was negatively associated with abundance of *R. ranae* (both $r_S \geq -0.50$, all $P \leq 0.05$). At Commune ($N = 18$ frogs), there was a single significant positive correlation between percent lymphocytes and abundance of *R. ranae* ($r_S = 0.48$, $P = 0.05$). In total then, we had 8 significant results when 7 were expected by chance (140 total correlations × α of 0.05 = 7). Finally, only 4 out of 60 correlations between frog size and blood parameters were significant within wetlands (3 significant expected by chance; results not shown). Thus, within wetlands, numbers of parasite–leukocyte and size–leukocyte correlations were not compelling.

Discussion

Our original hypothesis that pesticide levels affected nematode abundance in Northern Leopard Frogs, based on an earlier study in 2001 (King et al. 2008), was not supported by analysis of frogs collected in 2006. One wetland (Ag3 in King et al. 2008; Commune here) continued to have the highest abundances of *R. ranae*, but ranked abundances of the other nematodes had changed between 2001 and 2006. Thus, significant interannual variability in parasite communities was observed (also see Bennett et al. 1974; Kennedy 1993; Marcogliese et al. 2006; King et al. 2007).

When we analyzed all frogs together, we obtained dramatic evidence of significant, broad-scale patterns in host–parasite relationships, and of potential interspecific interactions between

Table 4. Correlations between Northern Leopard Frog (*Rana pipiens*) leukocyte profile values, nematode species richness, and abundance of three common nematodes ($N = 82$) for the pooled sample of frogs.

	Nematode species richness		*Oswaldocruzia* sp.		*Rhabdias ranae*		*Strongyloides* sp.	
Blood parameter	r_S	P	r_S	P	r_S	P	r_S	P
Neutrophil								
Percentage	**−0.25**	**0.02**	−0.06	0.57	**−0.23**	**0.03**	−0.09	0.37
Density	**0.22**	**0.05**	**0.27**	**0.01**	0.19	0.09	**0.27**	**0.01**
Eosinophil								
Percentage	0.21	0.06	**0.21**	**0.05**	**0.35**	**0.001**	0.15	0.18
Density	**0.47**	**<0.0001**	**0.39**	**0.0003**	**0.52**	**<0.0001**	**0.39**	**0.0003**
Lymphocyte								
Percentage	0.18	0.10	−0.01	0.95	0.13	0.26	0.06	0.57
Density	**0.47**	**<0.0001**	**0.26**	**0.02**	**0.38**	**0.0004**	**0.33**	**0.003**
Neutrophil:lymphocyte ratio	−0.21	0.06	−0.03	0.79	−0.18	0.11	−0.09	0.40

Note: Significant results are in boldface type.

Table 5. Comparison of nematode intensities between male and female Northern Leopard Frogs (*Rana pipiens*) within wetlands.

			Oswaldocruzia sp.		*Rhabdias ranae*		*Strongyloides* sp.	
Site	No. of females	No. of males	χ^2	P	χ^2	P	χ^2	P
Sauro	8	11	0.7	0.39	0.7	0.39	0.1	0.80
Rocher	11	20	2.4	0.12	1.2	0.27	1.6	0.21
Commune	17	14	**5.8**	**0.02**	0.4	0.54	3.3	0.07
Fairbanks	17	16	<0.1	0.85	**3.7**	**0.05**	**4.7**	**0.03**
Chibouet	14	18	3.1	0.08	<0.1	0.87	**5.5**	**0.02**

Note: Wetlands with lower pesticide concentrations appear first. Significant results are in boldface type.

Table 6. Analyses of coinfections within wetlands (sample sizes in Table 1).

Site	*Oswaldocruzia* sp. vs. *Rhabdias ranae*	*Oswaldocruzia* sp. vs. *Strongyloides* sp.	*Rhabdias ranae* vs. *Strongyloides* sp.
Sauro	No *R. ranae* present	0.60	No *R. ranae* present
Rocher	0.35	0.36	0.45
Commune	All frogs had both	All frogs had both	All frogs had both
Fairbanks	0.18	0.29	0.34
Chibouet	0.75	0.65	0.81

Note: Where tests were possible, shown are probabilities from Fisher's exact tests. Wetlands with lower pesticide concentrations appear first.

parasite species. However, those patterns were not significant at smaller (wetland) scales, and in many cases individual wetland patterns were opposite to those of the pooled sample. Specifically, correlations between nematodes and frog body size were significantly negative when frogs from five wetlands were pooled, but more often positive within wetlands. Similarly, significant evidence of associations between pairs of parasites (suggesting either synergy or antagonism) disappeared within wetlands. Finally, numerous correlations between leukocyte parameters and nematodes and size of host frogs in the pooled sample essentially were not observed within wetlands. Overall, the number of significant correlations obtained within wetlands was about what would be expected by chance with the number of tests performed (Rice 1989; also see Rothman 1990; Nakagawa 2004).

Among wetlands, sites producing smaller metamorphs may have had tadpoles that experienced greater competition during development (i.e., because of higher densities). These populations may have been less able to overcome nematode infections because higher density of hosts may have supported greater nematode transmission and smaller metamorphs may have had less developed immune systems. The same sorts of processes could have influenced nematodes themselves. The list of potential ecological influences on the relationships observed here is likely quite extensive (Schotthoefer et al. 2011).

We would not have detected the many patterns reported here had we used statistical approaches that initially included wetland as an explanatory variable, followed by dropping nonsignificant terms to produce final models (Crawley 2005). However, our data were not suited to complex parametric analysis. In many studies, organisms may be collected without recording study site or study site may not be included as a variable in analysis (e.g., either because of oversight or insufficient replication within study sites; McAlpine 1997; Shutler et al. 2009; Pulis et al. 2011). Our results illustrate that these cases may result in spurious conclusions about host–parasite associations and thus highlight the importance of including study site in statistical analyses (Weatherhead et al. 1991; Shutler et al. 1995, 1999, 2012; Marcogliese et al. 1996; Budischak et al. 2012).

The only compelling within-wetland pattern that we observed was the higher abundance of all three nematodes in Commune. It is difficult to ascribe this to pesticides, because only low atrazine and moderate metolachlor levels were observed at this site. It seems more likely that some other combination of environmental variables produced this pattern, but what those variables might be is not clear.

In general, the relationship between pesticides and various aspects of amphibian health are not straightforward (Marcogliese et al. 2009; Schotthoefer et al. 2011; Shutler and Marcogliese 2011);

wetland appears to be important, but characteristics of wetlands that produce the relationships observed are not known in our study area. King et al. (2007, 2010) and Schotthoefer et al. (2011) found a number of landscape correlates that predicted parasite communities in Northern Leopard Frogs and Bullfrogs (*Rana catesbeiana* Shaw, 1802 = *Lithobates catesbeianus* (Shaw, 1802)) and the latter authors also found additional evidence of a link between atrazine and likelihood of trematode parasitism; however, the patterns found in those studies primarily applied to helminths with complex life cycles (e.g., trematodes) and not directly transmitted nematodes.

Leukocyte data differed significantly among our study wetlands; these differences may have been related to parasites or pesticide exposures (also see Shutler and Marcogliese 2011). A significant literature links impaired immune function in frogs and other organisms with exposure to various contaminants, including pesticides (Hoole 1997; Carey et al. 1999, 2003; Voccia et al. 1999; Christin et al. 2003, 2004; Gilbertson et al. 2003). Curiously, we observed lower stress indices (neutrophil:lymphocyte ratios) in frogs exposed to higher herbicide concentrations, unlike in 2001 (King et al. 2008). However, these patterns may reflect size differences in frogs among wetlands that are associated with, for example, differences in population densities that were not quantified here and which may vary among years. Further evaluation of these pesticide–leukocyte relationships is warranted.

It seems unlikely that genetic differences among frogs from different wetlands could produce the extremes in nematode prevalence (from 0% to 100% in some cases) that we observed. Frog dispersal in many landscapes is sufficient to lead to relative panmixia at the scale that we studied (Austin et al. 2004; review in Smith and Green 2005). A more likely explanation for the extremes in nematode prevalence is variation in exposure (Shaw et al. 1998). Each of the nematode taxa that we examined has a direct life cycle (i.e., they are not transported by potentially mobile intermediate hosts). Thus, differences in frog exposure likely arise because of limited vagility of nematode parasites or to particular habitat characteristics which may promote viability and transmission of free-living infective stages, such as nutrients in the substrate (King et al. 2008; Weaver et al. 2010), leading to significant spatial variation in community structure (e.g., Kennedy et al. 1986; Gregory 1990). Chance events that lead to local extinctions may also be responsible.

Landscape ecology is a relatively new field that has been embraced by parasitologists (Taylor and Merriam 1996; Holt and Boulinier 2005; Biek and Real 2010; Sehgal 2010; Mlynarek et al. 2011). Despite the interest in landscape processes, however, there have been few empirical studies on amphibian parasites (but see Koprivnikar et al. 2006; King et al. 2007, 2010; Schotthoefer et al. 2011; Koprivnikar and Redfern 2012). Our study contributes to illustrating how spatial scale can influence the host–parasite relationships that we explored here. We have shown the importance of scale to interpreting our data, but there are additional challenges to identifying the causes of differences in parasite communities among wetlands.

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References

Apanius, V., Yorinks, N., Bermingham, E., and Ricklefs, R.E. 2000. Island and taxon effects in parasitism and resistance of Lesser Antillean birds. Ecology, **81**: 1959–1969. doi:10.1890/0012-9658(2000)081[1959:IATEIP]2.0.CO;2.

Austin, J.D., Lougheed, S.C., and Boag, P.T. 2004. Controlling for the effects of history and nonequilibrium conditions in gene flow estimates in northern bullfrog (*Rana catesbeiana*) populations. Genetics, **168**: 1491–1506. doi:10.1534/genetics.104.027987. PMID:15579701.

Baker, M.R. 1978. Morphology and taxonomy of *Rhabdias* spp. (Nematoda: Rhabdiasidae) from reptiles and amphibians of southern Ontario. Can. J. Zool. **56**(10): 2127–2141. doi:10.1139/z78-289.

Beadell, J.S., Atkins, C., Cashion, E., Jonker, M., and Fleischer, R.C. 2007. Immunological change in a parasite-impoverished environment: divergent signals from four island taxa. PLoS ONE, **2**(9): e896. doi:10.1371/journal.pone.0000896. PMID:17878931.

Ben Slimane, B., and Durette-Desset, M.C. 1997. Révision du genre *Oswaldocruzia* (Nematoda, Trichostrongylina, Molineoidea) en zone néartique avec description de cinq nouvelles espèces. Zoosystema, **19**: 61–79.

Bennett, G.F. 1970. Simple techniques for making avian blood smears. Can. J. Zool. **48**(3): 585–586. doi:10.1139/z70-098.

Bennett, G.F., Campbell, A.G., and Cameron, M. 1974. Hematozoa of passeriform birds from insular Newfoundland. Can. J. Zool. **52**(6): 765–772. doi:10.1139/z74-100. PMID:4209413.

Biek, R., and Real, L.A. 2010. The landscape genetics of infectious disease emergence and spread. Mol. Ecol. **19**: 3515–3531. doi:10.1111/j.1365-294X.2010.04679.x. PMID:20618897.

Boily, M.H., Bérubé, V.E., Spear, P.A., DeBlois, C., and Dassylva, N. 2005. Hepatic retinoids of bullfrogs in relation to agricultural pesticides. Environ. Toxicol. Chem. **24**: 1099–1106. doi:10.1897/04-126R.1. PMID:16110987.

Budischak, S.A., Jolles, A.E., and Ezenwa, V.O. 2012. Direct and indirect costs of co-infection in the wild: linking gastrointestinal parasite communities, host hematology, and immune function. Int. J. Parasitol. Parasites Wildl. **1**: 2–12. doi:10.1016/j.ijppaw.2012.10.001. PMID:24533308.

Bush, A.O., Lafferty, K.D., Lotz, J.M., and Shostak, A.W. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. **83**: 575–583. doi:10.2307/3284227.

Cabagna, M.C., Lajmanovich, R.C., Stringhini, G., Sanchez-Hernandez, J.C., and Pelzer, P.M. 2005. Hematological parameters of health status in the common toad *Bufo arenarum* in agroecosystems of Santa Fe Province, Argentina. Appl. Herpetol. **2**: 373–380. doi:10.1163/157075405774483085.

Canadian Council on Animal Care. 2003. Guidelines on the care and use of wildlife. Available from http://www.ccac.ca/Documents/Standards/Guidelines/Wildlife.pdf [accessed 1 May 2006].

Carey, C., and Bryant, C.J. 1995. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. Environ. Health Perspect. **103**(Suppl. 4): 13–17. doi:10.2307/3432406.

Carey, C., Cohen, N., and Rollins-Smith, L. 1999. Amphibian declines: an immunological perspective. Dev. Comp. Immunol. **23**: 459–472. doi:10.1016/S0145-305X(99)00028-2. PMID:10512457.

Carey, C., Bradford, D.F., Brunner, J.L., Collins, J.P., Davidson, E.W., Longcore, J.E., Ouellet, M., Pessier, A.P., and Schock, D.M. 2003. Biotic factors in amphibian population declines. *In* Amphibian decline: an integrated analysis of multiple stressor effects. *Edited by* G. Linder, S.K. Krest, and D.W. Sparling. SETAC Press, Pensacola, Fla. pp. 153–208.

CCME (Canadian Council of Ministers of the Environment). 2010. Canadian environmental quality guidelines summary table. Available from http://st-ts.ccme.ca/?chems510,136&chapters51 [accessed 20 October 2010].

Christin, M.S., Gendron, A.D., Brousseau, P., Ménard, L., Marcogliese, D.J., Cyr, D., Ruby, S., and Fournier, M. 2003. Effects of agricultural pesticides on the immune system of *Rana pipiens* and on its resistance to parasitic infection. Environ. Toxicol. Chem. **22**: 1127–1133. doi:10.1002/etc.5620220522. PMID:12729224.

Christin, M.S., Ménard, L., Gendron, A.D., Ruby, S., Cyr, D., Marcogliese, D.J., Rollins-Smith, L., and Fournier, M. 2004. Effects of agricultural pesticides on the immune system of *Xenopus laevis* and *Rana pipiens*. Aquat. Toxicol. **67**: 33–43. doi:10.1016/j.aquatox.2003.11.007. PMID:15019249.

Crawley, M.J. 2005. Statistics: an introduction using R. Wiley, West Sussex, England.

Davis, A.K., Maney, D.L., and Maerz, J.C. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol. **22**: 760–772. doi:10.1111/j.1365-2435.2008.01467.x.

Fallon, S.M., Bermingham, E., and Ricklefs, R.E. 2003. Island and taxon effects in parasitism revisited: avian malaria in the Lesser Antilles. Evolution, **57**: 606–615. doi:10.1554/0014-3820(2003)057[0606:IATEIP]2.0.CO;2. PMID:12703950.

Forbes, M.R., McRuer, D.L., and Shutler, D. 2006. White blood cell profiles of breeding American toads (*Bufo americanus*) relative to sex and body size. Comp. Clin. Pathol. **15**: 155–159. doi:10.1007/s00580-006-0623-5.

Forson, D.D., and Storfer, A. 2006. Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. Ecol. Appl. **16**: 2325–2332. doi:10.1890/1051-0761(2006)016[2325:AIRSIT]2.0.CO;2. PMID:17205907.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A.,

Lynch, J.D., Green, D.M., and Wheeler, W.C. 2006. The amphibian tree of life. Bull. Am. Mus. Nat. Hist. **297**: 1–370. doi:10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2.

Gendron, A.D., Marcogliese, D.J., Barbeau, S., Christin, M.-S., Brousseau, P., Ruby, S., Cyr, D., and Fournier, M. 2003. Exposure of leopard frogs to a pesticide mixture affects life history characteristics of the lungworm *Rhabdias ranae*. Oecologia, **135**: 469–476. PMID:12721838.

Gendron, A.D., Marcogliese, D.J., and Thomas, M. 2012. Invasive species are less parasitized than native competitors but for how long? The case of the round goby in the Great Lakes–St. Lawrence Basin. Biol. Invasions, **14**: 367–384. doi:10.1007/s10530-011-0083-y.

Gilbertson, M.K., Haffner, G.D., Drouillard, K.G., Albert, A., and Dixon, B. 2003. Immunosuppression in the northern leopard frog (*Rana pipiens*) induced by pesticide exposure. Environ. Toxicol. Chem. **22**: 101–110. doi:10.1002/etc.5620220113. PMID:12503752.

Gregory, R.D. 1990. Parasites and host geographic range as illustrated by waterfowl. Funct. Ecol. **4**: 645–654. doi:10.2307/2389732.

Gross, W.B., and Siegel, H.S. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. **27**: 972–979. doi:10.2307/1590198. PMID:6360120.

Hedrick, L.R. 1935. The life history and morphology of *Spiroxys contortus* (Rudolphi); Nematoda: Spiruridae. Trans. Am. Microsc. Soc. **54**: 307–335. doi:10.2307/3222323.

Holt, R., and Boulinier, T. 2005. Ecosystems and parasites: the spatial dimension. *In* Parasitism and ecosystems. *Edited by* F. Thomas, F. Renaud, and J.-F. Guégan. Oxford University Press, Oxford. pp. 68–84.

Hoole, D. 1997. The effect of pollutants on the immune response of fish: implications for helminth parasites. Parassitologia, **39**: 219–225. PMID:9802070.

Kennedy, C.R. 1993. The dynamics of intestinal helminth communities in eels *Anguilla anguilla* in a small stream: long term changes in richness and structure. Parasitology, **107**: 71–78. doi:10.1017/S0031182000079427.

Kennedy, C.R., Bush, A.O., and Aho, J.M. 1986. Patterns in helminth communities: why are birds and fish different? Parasitology, **93**: 205–215. doi:10.1017/S0031182000049945. PMID:3748613.

Kiesecker, J.M. 2002. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? Proc. Natl. Acad. Sci. U.S.A. **99**: 9900–9904. doi:10.1073/pnas.152098899. PMID:12118118.

King, K.C., McLaughlin, J.D., Gendron, A.D., Pauli, B.D., Giroux, I., Rondeau, B., Boily, M., Juneau, P., and Marcogliese, D.J. 2007. Impact of agriculture on the parasite communities of northern leopard frogs (*Rana pipiens*) in southern Quebec, Canada. Parasitology, **134**: 2063–2080. doi:10.1017/S0031182007003277. PMID:17672926.

King, K.C., Gendron, A.D., McLaughlin, J.D., Giroux, I., Brousseau, P., Cyr, D., Ruby, S.M., Fournier, M., and Marcogliese, D.J. 2008. Short-term seasonal changes in parasite community structure in northern leopard froglets (*Rana pipiens*) inhabiting agricultural wetlands. J. Parasitol. **94**: 13–22. doi:10.1645/GE-1233.1. PMID:18372616.

King, K.C., McLaughlin, J.D., Boily, M., and Marcogliese, D.J. 2010. Effects of agricultural landscape and pesticides on parasitism in native bullfrogs. Biol. Conserv. **143**: 302–310. doi:10.1016/j.biocon.2009.10.011.

Knutson, M.G., Richardson, W.B., Reineke, D.M., Gray, B.R., Parmelee, J.R., and Weick, S.E. 2004. Agricultural ponds support amphibian populations. Ecol. Appl. **14**: 669–684. doi:10.1890/02-5305.

Koprivnikar, J., and Redfern, J.C. 2012. Agricultural effects on amphibian parasitism: importance of general habitat perturbations and parasite life cycles. J. Wildl. Dis. **48**: 925–936. doi:10.7589/2011-09-258. PMID:23060494.

Koprivnikar, J., Baker, R.L., and Forbes, M.R. 2006. Environmental factors influencing trematode prevalence in grey tree frog (*Hyla versicolor*) tadpoles in southern Ontario. J. Parasitol. **92**: 997–1001. doi:10.1645/GE-771R.1. PMID:17152940.

Kuzmin, Y., Tkach, V.V., and Snyder, S.D. 2003. The nematode genus *Rhabdias* (Nematoda: Rhabdiasidae) from amphibians and reptiles of the Nearctic. Comp. Parasitol. **70**: 101–114. doi:10.1654/4075.

Lehman, C.M., and Williams, B.K. 2010. Effects of current-use pesticides on amphibians. *In* Ecotoxicology of amphibians and reptiles. 2nd ed. *Edited by* D.W. Sparling, G. Linder, C.A. Bishop, and S. Krest. SETAC Press, Pensacola, Fla. pp. 167–202.

Marcogliese, D.J., Boily, F., and Hammill, M.O. 1996. Distribution and abundance of stomach nematodes (Anisakidae) among grey seals (*Halichoerus grypus*) and harp seals (*Phoca groenlandica*) in the Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. **53**(12): 2829–2836. doi:10.1139/f96-243.

Marcogliese, D.J., Gendron, A.D., Plante, C., Fournier, M., and Cyr, D. 2006. Parasites of spottail shiners (*Notropis hudsonius*) in the St. Lawrence River: effects of municipal effluents and habitat. Can. J. Zool. **84**(10): 1461–1481. doi:10.1139/z06-088.

Marcogliese, D.J., King, K.C., Salo, H.M., Fournier, M., Brousseau, P., Spear, P., Champoux, L., McLaughlin, J.D., and Boily, M. 2009. Combined effects of agricultural activity and parasites on biomarkers in the bullfrog, *Rana catesbeiana*. Aquat. Toxicol. **91**: 126–134. doi:10.1016/j.aquatox.2008.10.001. PMID:19019467.

McAlpine, D.F. 1997. Helminth communities in bullfrogs (*Rana catesbeiana*), green frogs (*Rana clamitans*), and leopard frogs (*Rana pipiens*) from New Brunswick, Canada. Can. J. Zool. **75**(11): 1883–1890. doi:10.1139/z97-818.

McAlpine, D.F., and Burt, M.D.B. 1998. Helminths of bullfrogs, *Rana catesbeiana*, green frogs, *R. clamitans*, and leopard frogs, *R. pipiens* in New Brunswick. Can. Field-Nat. **112**: 50–64.

Mlynarek, J.J., Bert, D.G., Peralta-Vázquez, H., James, J.A., and Forbes, M.R. 2011. Relationships between gregarine infection in damselflies, wetland type, and landscape characteristics. Can. Entomol. **143**(6): 460–469. doi:10.4039/n11-028.

Nakagawa, S. 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav. Ecol. **15**: 1044–1045. doi:10.1093/beheco/arh107.

Norris, K., and Evans, M.A. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behav. Ecol. **11**: 19–26. doi:10.1093/beheco/11.1.19.

Pauly, G.B., Hillis, D.M., and Cannatella, D.C. 2009. Taxonomic freedom and the role of official lists of species names. Herpetologica, **65**: 115–128. doi:10.1655/08-031R1.1.

Pietrock, M., and Marcogliese, D.J. 2003. Free-living endohelminth stages: at the mercy of environmental conditions. Trends Parasitol. **19**: 293–299. doi:10.1016/S1471-4922(03)00117-X. PMID:12855379.

Poulin, R. 2007. Evolutionary ecology of parasites. Princeton University Press, Princeton, N.J.

Pulis, E.E., Tkach, V.V., and Newman, R.A. 2011. Helminth parasites of the wood frog, *Lithobates sylvaticus*, in prairie pothole wetlands of the northern great plains. Wetlands, **31**: 675–685. doi:10.1007/s13157-011-0183-6.

Raffel, T.R., Rohr, J.R., Kiesecker, J.M., and Hudson, P.J. 2006. Negative effects of changing temperature on amphibian immunity under field conditions. Funct. Ecol. **20**: 819–828. doi:10.1111/j.1365-2435.2006.01159.x.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution, **43**: 223–225. doi:10.2307/2409177.

Rohr, J.R., and McCoy, K.A. 2010. A qualitative meta-analysis reveals consistent effects of atrazine on freshwater fish and amphibians. Environ. Health Perspect. **118**: 20–32. PMID:20056568.

Rohr, J.R., Schotthoefer, A.M., Raffel, T.R., Carrick, H.J., Halstead, N., Hoverman, J.T., Johnson, C.M., Johnson, L.B., Lieske, C., Piwoni, M.D., Schoff, P.K., and Beasley, V.R. 2008. Agrochemicals increase trematode infections in a declining amphibian species. Nature, **455**: 1235–1239. doi:10.1038/nature07281. PMID:18972018.

Rothman, K.J. 1990. No adjustments are needed for multiple comparisons. Epidemiology, **1**: 43–46. doi:10.1097/00001648-199001000-00010. PMID:2081237.

Schotthoefer, A.M., Rohr, J.R., Cole, R.A., Koehler, A.V., Johnson, C.M., Johnson, L.B., and Beasley, V.R. 2011. Effects of wetland vs. landscape variables on parasite communities of *Rana pipiens*: links to anthropogenic factors. Ecol. Appl. **21**: 1257–1271. doi:10.1890/10-0374.1. PMID:21774428.

Sehgal, R.N.M. 2010. Deforestation and avian infectious diseases. J. Exp. Biol. **213**: 955–960. doi:10.1242/jeb.037663. PMID:20190120.

Shaw, D.J., Grenfell, B.T., and Dobson, A.P. 1998. Patterns of macroparasite aggregation in wildlife host populations. Parasitology, **117**: 597–610. doi:10.1017/S0031182098003448. PMID:9881385.

Shutler, D., and Marcogliese, D.J. 2011. Leukocyte profiles of Northern Leopard Frogs, *Lithobates pipiens*, exposed to pesticides and hematozoa in agricultural wetlands. Copeia, **2011**: 301–307. doi:10.1643/CP-10-065.

Shutler, D., Bennett, G.F., and Mullie, A. 1995. Sex proportions of *Haemoproteus* blood parasites and local mate competition. Proc. Natl. Acad. Sci. U.S.A. **92**: 6748–6752. doi:10.1073/pnas.92.15.6748. PMID:7624315.

Shutler, D., Alisauskas, R.T., and McLaughlin, J.D. 1999. Mass dynamics of the spleen and other organs in geese: measures of immune relationships to helminths? Can. J. Zool. **77**(3): 351–359. doi:10.1139/z98-226.

Shutler, D., Mullie, A., and Clark, R.G. 2004. Tree swallow reproductive investment, stress, and parasites. Can. J. Zool. **82**(3): 442–448. doi:10.1139/z04-016.

Shutler, D., Smith, T.G., and Robinson, S.R. 2009. Relationships between leukocytes and *Hepatozoon* spp. in green frogs, *Rana clamitans*. J. Wildl. Dis. **45**: 67–72.

Shutler, D., Alisauskas, R.T., and McLaughlin, J.D. 2012. Associations between body composition and helminths of lesser snow geese during winter and spring migration. Int. J. Parasitol. **42**: 755–760. doi:10.1016/j.ijpara.2012.05.008. PMID:22713759.

Smith, M.A., and Green, D.M. 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? Ecography, **28**: 110–128. doi:10.1111/j.0906-7590.2005.04042.x.

Speare, R. 1989. Identification of species of *Strongyloides*. *In* Strongyloidiasis: a major roundworm infection of man. *Edited by* D.I. Grove. Taylor and Francis, London. pp. 11–84.

Stuart, B.L. 2008. The phylogenetic problem of *Huia* (Amphibia: Ranidae). Mol. Phylogenet. Evol. **46**: 49–60. doi:10.1016/j.ympev.2007.09.016. PMID:18042407.

Taylor, P.D., and Merriam, G. 1996. Habitat fragmentation and parasitism of a forest damselfly. Landsc. Ecol. **11**: 181–189. doi:10.1007/BF02447516.

Vanderburgh, D.J., and Anderson, R.C. 1987. The relationship between nematodes of the genus *Cosmocercoides* Wilkie, 1930 (Nematoda: Cosomcercoidea) in toads (*Bufo americanus*) and slugs (*Deroceras laeve*). Can. J. Zool. **65**(7): 1650–1661. doi:10.1139/z87-254.

Vleck, C.M., Vertalino, N., Vleck, D., and Bucher, T.L. 2000. Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adélie penguins. Condor, **102**: 392–400. doi:10.2307/1369652.

Voccia, I., Blakeley, B., Brousseau, P., and Fournier, M. 1999. Immunotoxicity

of pesticides: a review. Toxicol. Ind. Health, **15**: 119–132. doi:10.1191/074823399678846637.

Weatherhead, P.J., Bennett, G.F., and Shutler, D. 1991. Sexual selection and parasites in wood-warblers. Auk, **108**: 147–152.

Weaver, H.J., Hawdon, J.M., and Hoberg, E.P. 2010. Soil-transmitted helminthiases: implications of climate change and human behavior. Trends Parasitol. **26**: 574–581. doi:10.1016/j.pt.2010.06.009. PMID:20580609.

Wilson, K., Bjornstad, O.N., Dobson, A.P., Merler, S., Poglayen, G., Randolph, S.E., Read, A.F., and Skorping, A. 2001. Heterogeneities in macroparasite infections: patterns and processes. *In* The ecology of wildlife diseases. *Edited by* P.J. Hudson, A. Rizzoli, B.T. Grenfell, H. Heesterbeck, and A.P. Dobson. Oxford University Press, Oxford. pp. 6–44.

Wright, K.M. 2001. Amphibian hematology. *In* Amphibian medicine and captive husbandry. *Edited by* K.M. Wright and B.R. Whitaker. Krieger, Malabar, Fla. pp. 129–146.