Parasite communities of leopard frogs (*Rana pipiens*) from wetland habitats impacted by agriculture

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Abstract

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Mounting empirical evidence links environmental disturbance with changes in parasitism. Agricultural run-off in wetland habitats and surrounding land use can greatly impact parasite acquisition by affecting hosts or the parasites themselves. Thus, the aim of this thesis was to determine if agricultural impacts were detectable in the parasite communities of frogs. Parasite communities of metamorphic *Rana pipiens* from five reference wetlands and four wetlands receiving agricultural run-off in Quebec's St. Lawrence River basin were examined in 2004 and 2005. Parc Le Rocher, a reference wetland within a managed park, and Rivière Chibouet, an agricultural wetland with heavy pesticide contamination, differed from the other wetlands perhaps because of surrounding agriculture.

The component and infracommunities in frogs from these wetlands were characterised by low species richness and diversity. Spearman-rank correlations and a multivariate analysis revealed that the landscape variables, particularly agricultural area, surrounding the wetland, and concentrations of dissolved organic carbon were positively related to parasite community structure. Landscape effects may hinder parasite transmission by limiting the access of definitive hosts to particular wetlands. *Echinostoma* sp. was the only parasite found at all localities in both years of collection. Abundance of this parasite was positively associated with agricultural area, as well as

with Parc Le Rocher and Rivière Chibouet, suggesting that generalist parasites persist in impacted environments compared to more host-specific species. At Rivière Chibouet, pesticide contamination may have contributed to reduced parasitism, possibly through negative effects on intermediate hosts and free-living stages. This study suggests that the parasite communities of leopard frogs reflect environmental disturbance, and may be appropriate sentinel organisms for the ecological condition of wetland habitats.

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LITERATURE REVIEW

Parasite ecology

Parasitism is the dominant lifestyle on Earth, and over fifty-percent of all organisms are parasitic in at least one stage of their life cycle (Price, 1980; Poulin and Morand, 2004). Parasites are ubiquitous in aquatic and terrestrial ecosystems and infect virtually every animal and plant species. Individuals may be infected by a single parasite, a population of a particular parasite species, or more often, by a whole community of different species (Bush *et al.,* 1997). The presence of a particular parasite in its host is due to the shared evolutionary history of the two organisms (Janovy *et al.*, 1992; Poulin, 1997), whereas the development of parasite communities may result from interactions among parasite species and the availability of vacant niches in the host species over evolutionary time (Price, 1987). However, the composition and structure of the parasite community also depends upon local ecological conditions, such as host and parasite life-histories and the abiotic features of the environment (Goater *et al.*, 1987).

Parasites display a diversity of life-history patterns. These range from those using a single host to those that require two or more different host species to complete their life cycle (Poulin, 1998). Parasites that require only one host for development and transmission are considered to have direct life cycles. Monogeneans, some copepod and many nematode species are examples of such parasites. Parasite species that require two or more host species to complete their life cycles, have indirect life cycles. Digeneans, cestodes, acanthocephalans, and some nematodes are examples of parasitic helminths that have indirect life cycles.

Transmission between hosts can occur either actively or passively (Esch and Fernandez, 1993). In the case of active transmission, parasite larvae free in the environment seek a host and incur a direct energy cost; these parasites usually penetrate the host, often within a temporally constrained infection window. Alternatively, passive transmission does not require the larval stage to expend energy in the transfer to a host, but an energy cost is incurred in preparation for transmission; a host becomes infected passively through the ingestion of infective stages, usually through the food chain. Ultimately, parasite transmission hinges on the availability of infected and uninfected hosts, the survival of all life cycle stages (parasitic and free-living), temporal and spatial overlap of hosts and parasite infective stages, and in most cases, the trophic interactions between hosts (Marcogliese and Cone, 1997a; Marcogliese, 2001, 2003). Because the presence or absence of a single parasite species can reflect the presence or absence of intermediate and definitive host species in a given habitat (Hechinger and Lafferty, 2005), parasites reflect the biodiversity of the habitat and can be used to trace food-web relationships (Marcogliese, 2001, 2003, 2004). Therefore, "healthy" ecosystems are considered to have a diverse parasite fauna (Marcogliese, 2005; Hudson *et al.,* 2006).

Amphibians occupy two positions in food webs by concurrently functioning as predator of aquatic animals and prey for terrestrial animals. Because they link the aquatic and terrestrial habitats, organisms in the middle of the food web are thought to be better suited as biodiversity indicators (Marcogliese, 2004). This places them in an intermediate trophic level where they simultaneously serve as intermediate hosts for some parasites and definitive hosts for others. However, the development of parasite communities in

amphibians is not solely dependent upon food web dynamics (Aho, 1990) as parasite species with direct life cycles are also common.

The species richness of a community is influenced by a variety of host attributes. Kennedy *et al.* (1986) proposed that the complexity of the host's alimentary canal, the ability of the host to freely select from a broad range of prey species, host vagility, and host physiology influence the complexity and size of the overall parasite community. Mammals and birds are hosts to a broad diversity of parasite species, but relatively depauperate parasite communities are thought to exist in fishes and amphibians (Aho, 1990). Although amphibians are generally opportunistic and gape-limited feeders that feed on a variety of prey species, the diversity of their parasite communities is theoretically constrained by their restricted ability to migrate, their simple digestive system, and an ectothermic metabolism (Kennedy *et al.*, 1986; Goater *et al.,* 1987; Aho, 1990). Aho (1990) determined that the average infracommunity species richness and average parasite abundance (with ranges in parentheses) in amphibians were 0.80 (0-2.5) and 7.83 (0-83.1), respectively. A number of more recent studies, however, have shown that these values may underestimate species richness and average parasite abundance in frogs from temperate (Muzzall, 1991a; McAlpine, 1997; McAlpine and Burt, 1998; Gillilland and Muzzall, 1999) and tropical habitats (Barton, 1999; Paredes-Calderón *et al.,* 2004; Luque *et al.,* 2005).

Surveys of helminth parasites of leopard frogs in North America have generally found that few if any of the parasites infecting them are host specific. In fact, most of the parasite species infecting leopard frogs are shared among ranid species (McAlpine, 1997). The relative dominance of individual parasite species and life cycle stages vary

Table 1 – List of parasite species found infecting *Rana pipiens* in this study, their known intermediate and definitive hosts in north-eastern North America, and their mode of transmission to *R. pipiens*.

Class	Parasite	Definitive Host	First Intermediate Host	Second Intermediate Host	Mode of transmission to *R. pipiens*
DIGENEA	*Alaria* sp.	Mammalia: *Canis latrans*[1, 2], *Vulpes vulpes*[2, 3, 4]	Gastropoda: *Planorbula* sp.[5], *Promenetus* sp.[5]	Anura: *Bufo americanus*[6], *Rana catesbeiana*[7], *Rana clamitans*[7], ***Rana pipiens***[7], *Rana sylvatica*[6],	Skin Penetration
	Apharyngostrigea pipientis	Aves: *Ardea* sp.[5], *Columba livia*[8] *Butorides virescens*[5]	Gastropoda: *Planorbula* sp.[5]	Anura: *Hyla versicolor*[6], *R. catesbeiana*[7], *R. clamitans*[7], ***R. pipiens***[7]	Skin Penetration
	Clinostomum sp.	Aves: Ardeidae[5]	Gastropoda: *Helisoma* spp.[6], *Lymnaea* sp.[6]	Anura: *R. catesbeiana*[7], *R. clamitans melanota*[9], *R. clamitans*[10] ***R. pipiens***[11] Caudata: *Notophthalmus viridescens*[12] Osteichthyes: *Perca flavescens*[13]	Skin Penetration
	Diplostomum spp.	Aves[5]	Gastropoda: *Succinea* sp.[5], *Lymnaea* sp.[14]	Anura: ***R. pipiens,*** *R. catebeiana*[14] Caudata: *Necturus maculosus*[14] Osteichthyes[13]	Skin Penetration

Class	Parasite	Definitive Host	First Intermediate Host	Second Intermediate Host	Mode of transmission to *R. pipiens*
	Echinostoma spp.	Aves[8]: *Actitis hypoleucos, Anas* spp., *Anser* spp., *Aythya* spp., *Branta* spp., *Buchephala clangula, Buteo lagopus, Columba livia, Corvus* spp., *Cygnus* spp., *Dendrocygna viduata, Haematopus ostraleugs, Hirundo rustica, Larus ridibundus, Melanitta* spp., *Meleagris gallopavo, Mergus* spp., *Motacilla flava, Neochen jubatus, Numenius arquata, Nycticorax nycticorax, Oxyura jamaicensis, Phasianus colchicus, Perdix perdix, Rissa tridactyla, Scolopax rusticola, Streptopelia* spp., *Tadorna ferruginea, Vanellus vanellus* Mammalia: *Castor canadensis,*[16] *Martes pennanti*[17], *Ondatra zibethicus*[4,15,16,17], *Vulpes vulpes*[4]	Gastropoda[8]: *Amerianna* spp., *Anisus* spp., *Biomphalaria contortus, Helisoma trivolvis, Lymnaea* spp., *Planorbarius* spp., *Planorbis* spp., *Physa* spp.,	Anura: *B. americanus*[8, 19], *Pseudacris crucifer*[15], *R. catesbeiana*[7], *R. c. melanota*[21], *R. clamitans*[7], ***R. pipiens***[7, 8], *R. sylvatica*[21] Caudata: *Ambystoma laterale*[22] Gastropoda[8]: *Anisus* spp., *Bithynia* spp., *Lymnaea* spp., *Physa* spp., *Planorbis* spp., *Sphaerium* sp.	Skin Penetration
	Fibricola sp.	Mammalia: *Mephitis mephitis*[23]	Gastropoda: *Physa* sp.[6,24]	Anura: *P. crucifer*[25], ***R. pipiens,*** *R. sylvatica*[25]	Skin Penetration
	Glypthelmins quieta	Anura: *R. catesbeiana*[7,26], *R. c. melanota*[9,11,26,27], *R. clamitans*[7,10], ***R. pipiens***[7,11], *R. sylvatica*[28]	Gastropoda: *Helisoma* spp.[6], *Physa* sp.[29]	-	Skin Penetration / Ingestion
	Gorgoderina attenuata	Anura: *R. catesbeiana*[7,26], *R. c. melanota*[9,26], *R. clamitans*[7], ***R. pipiens***[7]	Gastropoda: *Sphaerium* sp.[5]	Anura: *Rana* tadpoles[30], ***Rana pipiens***[30], odonate larvae[31], caddisfly and beetle larvae[31], crayfish[31]	Skin Penetration / Ingestion

Class	Parasite	Definitive Host	First Intermediate Host	Second Intermediate Host	Mode of transmission to *R. pipiens*
	Haematoloechus varioplexus	Anura: *P. crucifer*[25], *R. catesbeiana*[7], *R. c. melanota*[26], *R. clamitans*[7], ***R. pipiens***[7], *R. sylvatica*[25]	Gastropoda: *Planorbula* sp.[5]	Insecta: dragonfly nymphs (*Sympetrum*)[5,6]	Ingestion
	Haematoloechus medioplexus	Anura: *B. americanus*[32] *R. catesbeiana*[7], *R. c. melanota*[26], *R. clamitans*[7], ***R. pipiens***[7, 26, 32]	Gastropoda: *Planorbula* sp.[5]	Insecta: dragonfly nymphs (*Sympetrum*)[5,6]	Ingestion
	Halipegus sp.	Anura: *R. catesbeiana*[7, 12], *R. c. melanota*[28], *R. clamitans*[7,10], ***R. pipiens***[7,] Caudata: *Notophthalmus viridescens*[33]	Gastropoda: *Physa* sp.[29], *Helisoma* sp.[29]	Copepoda: *Cyclops* sp.[29], *Mesocyclops* sp.[29] Insecta: dragonfly nymphs[33, 34] Ostracoda[35]	Ingestion
NEMATODA	*Cosmocercoides dukae*	Anura: *P. triseriata*[29], *R. catesbeiana*[7], *R. clamitans*[7, 10], ***R. pipiens***[7, 11], *R. sylvatica*[25, 27]	Gastropoda: *Deroceras* sp.[36]	-	Skin Penetration
	Oswaldocruzia sp.	Anura: R. catesbeiana[7], R. clamitans[7, 10], ***R. pipiens***[7,11]	-	-	Skin Penetration
	Rhabdias ranae	Anura: *P. crucifer*[19, 25], *R. c. melanota*[27, 28], *R. clamitans*[7], ***R. pipiens***[7,11] Caudata: *Amystoma laterale*[22]	-	-	Skin Penetration
	Spiroxys sp.	Reptilia: *Apalone s. spinifera*[37], *Chelydra s. serpentine*[37], *Chrysemys picta*[38], *Clemmys guttata*[37], *Emydoidea blandingi*[37], *Graptemys geographica*[37], *Sternotherus odoratus*[37], *Terrapene c. carolini*[37]	Copepoda: *Cyclops* sp.[37], *Mesocyclops* sp.[37]	Anura: *P. crucifer*[28], *R. catesbeiana*[7,26], *R. c. melanota*[11,26], *R. clamitans*[7], ***R. pipiens***[7,11], *R. sylvatica*[28] Caudata: *N. viridescens*[12, 39] *A. laterale*[22]	Ingestion

Class	Parasite	Definitive Host	First Intermediate Host	Second Intermediate Host	Mode of transmission to *R. pipiens*
	Strongyloides sp.	Anura: *R. catesbeiana*[40], ***R. pipiens***[40] Mammalia: *Bos taurus*[41], *Ovis aries*[42], *Canis familiaris*[43], *Equus caballus*[44], *Felis catus*[45], *Sus scrofa*[45, 46]	-	-	Skin Penetration
CESTODA	*Proteocephalus* sp.	Anura: *R. catesbeiana*[7], *R. clamitans*[7, 10], ***R. pipiens***[7]	Crustacea[47]	-	Ingestion

1. Pearson, 1956 2. Pearson, 1954 3. Law & Kennedy, 1932 4. Smith, 1978 5. Yamaguti, 1971 6. Yamaguti, 1975
7. MacAlpine & Burt, 1998 8. McDonald, 1969 9. Muzzall *et al.*, 2001 10. Yoder *et al.*, 2001 11. Gillilland & Muzzall, 1999
12. Muzzall, 1991a 13. Gibson, 1996 14. Marcogliese *et al.*, 2000 15. Smith & Archibald, 1967 16. Anonymous, 1930
17. Gupta, 1962 18. MacKinnon & Burt, 1978 19. Bolek & Coggins, 2000 21. Najarian, 1955 22. Muzzall & Schinderle, 1992
23. Webster & Wolfgang, 1956 24. Dubois, 1968, 1970 25. Yoder & Coggins, 1996 26. Muzzall, 1991b 27. Bolek & Coggins, 2001 28. Muzzall & Peebles, 1991 29. Schell, 1970 30. Rankin, 1939 31. Prudhoe & Bray, 1982 32. Fortner, 1923 33. Goater *et al.*, 1990 34. Zelmer & Esch, 1998a 35. Zelmer & Esch, 1998b 36. Vanderburgh & Anderson, 1986 37. Hedrick, 1935
38. Esch & Gibbons, 1967 39. Muzzall *et al.*, 2003 40. Rau *et al.*, 1978 41. Frechette & Gibbs, 1971 42. Ayalew *et al.*, 1973
43. Seah *et al.*, 1975 44. Slocombe & McCraw, 1973 45. Slocombe, 1973a 46. Slocombe, 1973b 47. Schmidt, 1970

among communities (McAlpine, 1997; McAlpine and Burt, 1998). As shown in Table 1, the adult parasite community in leopard frogs is dominated by digenetic trematodes. A few species of nematodes, cestodes, and acanthocephalans have also been reported, though the latter two are rarely encountered (McAlpine and Burt, 1998). Most infections of adult trematodes establish after larval stages are ingested along with invertebrate (e.g. insect larvae) or vertebrate prey (e.g. tadpoles). A number of nematodes, including *Cosmocercoides dukae*, *Oswaldocruzia* sp., *Rhabdias ranae,* and *Strongyloides* sp. infect the host by cutaneous penetration and develop to adulthood. Other parasites, including the digenetic trematodes *Glypthelmins quieta* and *Gorgoderina attenuata* use frogs as intermediate and definitive hosts, and are acquired either by penetration or ingestion with prey (Yamaguti, 1975). *Glypthelmins quieta* encyst in the skin of frogs, and then develop to adulthood in the intestine after being swallowed along with sloughed skin. *Gorgoderina attenuata* can be found as metacercariae in aquatic invertebrates and also in the body cavity of tadpoles and metamorph frogs. This parasite species develops into adulthood when frogs ingest invertebrate prey or other infected frogs. Ultimately, adult parasites infecting amphibians typically establish in one of four main sites: the lungs (*Haematoloechus* spp. and *R. ranae*), buccal cavity (*Halipegus occidualis*), urinary bladder (*G. attenuata*), and intestine (*G. quieta, C. dukae, Oswaldocruzia* sp., *Strongyloides* sp.) (McAlpine and Burt, 1998).

The larval parasite community in leopard frogs is composed mainly of species of digenean metacercariae, but larval nematodes, such as *Spiroxys* sp., are also present (McAlpine and Burt, 1998). Tissue penetration appears to be the predominant mode of infection for parasites that use frogs as intermediate hosts. Exceptions include *Spiroxys*

sp. and other nematodes of the order Spirurida which are typically ingested (Hedrick, 1935). Larval parasites encyst in specific sites. For example, *Echinostoma* spp., the most widely occurring parasites of frogs, encyst in the kidney (Olsen, 1974). Others, including *Apharyngostrigea pipientis* and Gorgoderidae gen. sp. are found loosely encysted inside the body cavity (e.g. McAlpine, 1997, McAlpine and Burt, 1998). A number of maetacercariae, including those of *Alaria* sp., *Clinostomum* sp., and *Fibricola* sp. as well as larvae of *Spiroxys* sp. infect the leg and body musculature (McAlpine and Burt, 1998; Gillilland and Muzzall, 1999). In addition, *Diplostomum* spp., frequent parasites of fishes in the St. Lawrence River (Marcogliese *et al.,* 2001a), have been found in the lens of mudpuppies and bullfrog tadpoles (Marcogliese *et al.,* 2000).

Habitat disturbance and parasite communities

In theory, the spread of an infectious agent through a population increases with the density and availability of susceptible and infected hosts (Anderson and May, 1986; Arneberg *et al.,* 1998). Thus, parasite population density and community structure may be affected if an environmental disturbance has a negative effect on the density and/or accessibility of intermediate and definitive hosts.

Drastic consequences may result for the host and parasites when there are sudden modifications of the ecological context within which they interact (Scott, 1988; Lafferty and Kuris, 1999). Habitat disturbance can come in many forms. It can be pervasive, and may impede or enhance parasite transmission in the ecosystem. There is mounting experimental and field evidence which demonstrates that a variety of types of environmental disturbance (e.g. pollution) can influence the formation of parasite communities and the abundances of individual parasite species (Poulin, 1992; Lafferty,

1997; MacKenzie, 1999; Marcogliese, 2004, 2005). Disturbance can produce indirect or direct effects on (1) the intermediate host populations (2) the definitive host populations (3) parasitic and free-living stages, or (4) the susceptibility of hosts to infection.

The impact of environmental contaminants on invertebrate intermediate host populations can alter parasite populations and communities in other hosts. Mortality, shifts in the species composition, and reduced species diversity of freshwater invertebrate communities are all consequences of aquatic pollutants, including agricultural insecticides (Schulz and Liess, 1999; Clements *et al.,* 2000, Relyea, 2005). The reproduction and lifespan of many invertebrates (e.g. planktonic crustaceans, insects, gastropods) are reduced when contaminants such as pesticides (Streit and Peter, 1978; Schluz and Liess, 1999; Perveen, 2000; Rohr and Crumrine, 2005; Sandland and Carmosini, 2006), endocrine-modulating hormones (Czech *et al.,* 2001), and heavy metals (Mance, 1987) are present in the aquatic environment. In addition to being directly impacted by contaminants, invertebrate communities can be indirectly affected. Dewey (1986) found the herbicide atrazine caused a trophic cascade effect on aquatic insect communities through a reduction of food (periphyton, macrophytes) for herbivorous insects. Overall, the occurrence of indirect life cycle parasites may decrease in polluted habitats if intermediate host populations decline or if infected hosts die at a faster rate than uninfected hosts (Guth *et al.,* 1977; Brown and Pascoe, 1989; Lafferty and Kuris, 1999).

When concentrations of aquatic nutrients and elements deviate from normal levels, intermediate hosts may be affected and shifts in the structure of the parasite community can result. For example, Marcogliese and Cone (1996, 1997b) reported that

acidification from acid precipitation caused a decrease in digenean infections in American eels (*Anguilla rostrata*) in Nova Scotian rivers. This decrease was attributed to a reduction in the density of snail hosts due to their sensitivity to acidic conditions (Cone *et al.,* 1993). A similar result was detected in the digenean communities of perch *(Perca fluviatilis*) in lakes and acidic reservoirs in Finland (Halmetoja *et al.,* 2000).

An elevated level of nutrients, particularly phosphorus, can cause eutrophication in freshwater ecosystems. Subsequent increases in primary productivity among macrophytes and periphyton augment food supply for invertebrates (Sankurathri and Holmes, 1976) thereby promoting the growth of populations and the parasites they transmit (Lafferty and Kuris, 1999). Field studies have demonstrated that increases in total parasite species abundance can result from influxes of nutrients from runoff sources associated with agriculture (Johnson and Chase, 2004) and from point sources such as urban effluents (Marcogliese and Cone, 2001; Coyner *et al.,* 2003). Johnson and Chase (2004) demonstrated that in eutrophic wetlands exposed to agricultural runoff, the snail community composition shifted towards *Planorbella* spp., the first intermediate host of the digenetic trematode *Ribeiroia ontradae*. As this parasite has been linked with limb and skeletal malformations in amphibians (Johnson *et al.*, 1999, 2001), Johnson and Chase (2004) suggested that eutrophication may be facilitating this "disease epidemic." Parasites of fishes that incorporate oligochaetes in their life cycles, such as myxozoans (Marcogliese and Cone, 2001) and the nematode *Eustrongyloides* spp. (Coyner *et al.,* 2003), are particularly abundant in water enriched by urban effluent.

The final stages of eutrophication, or hyper-eutrophication, can lead to the impoverishment of intermediate host communities due to anoxia in the sediments and the

dominance of monospecific algal blooms. As a consequence, parasite communities become depauperate due to a decline of benthic invertebrates (Zander and Reimer, 2002). Parasite communities in these environments shift to ones dominated by species transmitted by planktonic intermediate hosts (e.g. cestodes and some digeneans) rather than benthic ones, and may display a reduced number of specialist parasites, and a predominance of parasites with direct life cycles (e.g. copepods and monogeneans) (Overstreet and Howse, 1977; Zander, 1998).

Little is known of how disturbance of the surrounding terrestrial habitat can affect invertebrates and parasite transmission within aquatic ecosystems. However, a decrease in aquatic invertebrate species richness was found in freshwater streams with a reduced vegetation margin (Moore and Palmer, 2005), and Marcogliese *et al.* (2001b) concluded that a reduction of aquatic invertebrate hosts, as a result of local deforestation, may have caused a decrease in the parasite infracommunity species richness of redbelly dace (*Phoxinus eos*) in boreal lakes.

Definitive hosts must also be present in a habitat for parasites to complete their life cycle and continue infection of intermediate host populations. Essentially, low infection prevalence in intermediate host populations is expected when definitive hosts are scarce. Heavier infections and an increased probability of transmission are expected when they are abundant (Zander and Reimer, 2002). Birds and small carnivorous mammals are among the major definitive hosts of larval parasites in amphibians, and their use of a habitat decreases as it deteriorates. Unsuitable habitats may have fewer prey items (Miles *et al.*, 2002; Niemi *et al.*, 1999) forcing terrestrial hosts to forage elsewhere, or they may be fragmented and disturbed by anthropogenic land use limiting the access of

hosts to the area (Kuris and Lafferty, 1994; Huspeni and Lafferty, 2004; Koprivnikar *et al.,* 2006a). For example, Koprivnikar *et al.* (2006a) reported that forest cover surrounding wetland habitats was positively associated with the prevalence of *Alaria* sp. infecting *Hyla versicolor* tadpoles. They suggested that deforested habitats were unsuitable for the canid definitive hosts of *Alaria* sp.

Definitive hosts have a "downstream" influence on the composition of parasite communities in intermediate hosts (Combes, 2001; Hechinger and Lafferty, 2005). Esch (1971) demonstrated that the ratio between larval and adult stages comprising the parasite communities of centrarchid fishes differed between eutrophic and oligotrophic lakes due to the increased use by avian predators of the more productive lakes. Fishes from eutrophic lakes were reported to have a greater abundance of larval parasite stages that infect birds. More recently, Huspeni and Lafferty (2004) found that prevalence and species richness of trematodes in snails increased after a saltmarsh was restored. The changes in the parasite communities were attributed directly to an increase in avian use of the restored habitat.

Amphibian species richness in a wetland habitat may influence the diversity of parasite species using them as definitive hosts. Factors that affect the access of particular amphibian species or the number of individuals to wetlands may reduce parasite species richness by limiting the opportunity for exchange of parasites among different individuals and species. Such factors include reductions in forest cover and marsh habitat, which have been linked to decreases in amphibian abundance, and increases in road density and poor water quality, which are associated with reduced amphibian species richness (Houlahan and Findlay, 2003). Similarly, high nitrogen and phosphorus concentrations

associated with fertilizer run-off can cause a reduction in amphibian species richness in agricultural ponds (Knutson *et al.,* 2003).

Parasites may also be directly affected by pollutants. For example, pollutants can cause a reduction of longevity and infectivity of parasite free-living stages (Pietrock and Marcogliese, 2003). Effects on survival and transmission of infective stages, however, differ among species, the contaminants and concentrations. Mortality of free-living parasite larvae occurs when natural environmental variables (e.g. pH, dissolved oxygen concentration, temperature, and salinity) deviate from normal levels or when pollutants (e.g. heavy metals, PCBs, and pesticides) are introduced into the environment (MacKenzie *et al.,* 1995; Pietrock and Marcogliese, 2003). Various pesticides kill parasite eggs (Guttowa and Boniecka, 1975, 1976), digenean miracidia (Guttowa, 1975; Tchounwou, 1991) and cercariae (Ghandour and Webbe, 1975; Koprivnikar *et al.,* 2006b), and cestode coracidia (Guttowa, 1975). Moreover, toxic substances leaching from agricultural lands may have nematicidal properties (Kimpinksi *et al.,* 1998). Thus, pesticides that harm the free-living stages of parasites directly have the potential to significantly reduce parasite abundance and alter the diversity of the parasite community.

Endoparasites that have already established in the host may also experience direct damage from contaminants in the host environment. For example, parasites in the alimentary tract may come into direct contact with contaminants when food or water is ingested (Khan and Thulin, 1991). In contrast, directly-transmitted ectoparasites, including monogeneans and protozoans, that are in constant contact with the external environment appear unaffected by contamination and may be more abundant in pollutant environments (Skinner, 1982; Yeomans *et al.,* 1997). Indeed, a high prevalence and

abundance of directly transmitted parasites are thought to reflect a contaminated environment and impaired host immunity (Khan and Thulin, 1991; MacKenzie *et al.*, 1995; Landsberg *et al.*, 1998).

Agriculture, parasites, and the amphibian immune system

North American agriculture relies heavily on the use of pesticides to treat cultivated land. These chemical cocktails can be comprised of herbicides such as atrazine used in controlling weeds in corn production; insecticides, used to kill insects; and fungicides, used to prevent loss in crops caused by parasitic fungi (Lean, 2000). These pesticides eventually leach into the groundwater and enter aquatic ecosystems. This is particularly true of atrazine, a commonly used pesticide which is hydrophilic and highly mobile in soil under wet conditions (Lemieux *et al.*, 1995). Pesticides and fertilizers are often applied in spring, and short term pulses of these contaminants enter wetland habitat during the periods of amphibian breeding and development. Thus, the concentrations of agricultural run-off are relatively high during the most vulnerable stages in an amphibian's ontogeny and, in particular, when their immune system is developing (Gilbertson *et al.*, 2003; Carey and Bryant, 1995). Not only can amphibian development (de Solla *et al.*, 2002; McDaniel *et al.*, 2004; Relyea, 2004a), hatching success (de Solla *et al.*, 2002), locomotion (Bridges, 1999; Bridges and Semlitsch, 2000) and predator avoidance behaviour (Cooke, 1981; Carey and Bryant, 1995) be affected pesticides, but so can their susceptibility to opportunistic pathogens and parasites (Carey and Bryant, 1995; Taylor *et al.*, 1999; Kiesecker, 2002; Christin *et al.*, 2003; Gendron *et al.*, 2003; Gilbertson *et al.*, 2003; Belden and Kiesecker, 2005).

Toxicological research on amphibians is limited compared with that on other vertebrates. However, several immunological, histological, molecular, biochemical and life-history biomarkers have been established to act as surrogate measures of how and to what extent amphibians are affected by pollution (see review by Venturino *et al.,* 2003). Various studies have shown that amphibians exposed to pesticides experience a variety of sublethal effects such as a suppression of antibody responses (Gilbertson *et al.,* 2003), a temporary decrease in lymphocyte proliferation (Christin *et al.*, 2003; Christin *et al.*, 2004), and progressive gonadal degeneration (Hayes *et al.,* 2002), to name just a few.

Pesticides have been demonstrated to cause immunotoxicity in a range of aquatic taxa, particularly fishes (Hoole, 1997; Vocia *et al.,* 1999). They can act as immunosuppressive agents by altering the innate or adaptive immune components of the immune system (Gilbertson *et al.*, 2003). It follows that damage to the host's immune system may result in a greater acquisition of pathogens or parasites (Snieszko, 1974; Carey, 1993; Carey *et al.,* 1999). Few studies, however, have specifically examined the impact of pollutants on the resistance of anurans to pathogens and parasites. Christin *et al.* (2003) demonstrated experimentally that pesticide combinations and exposure levels comparable to those found in nature can impair the immune response of leopard frogs and lead to an increased prevalence of the nematode *Rhabdias ranae* in the lungs. Furthermore, Kiesecker (2002) demonstrated through a combination of laboratory exposures and field manipulations that immunosuppression from pesticide exposure increased the susceptibility of wood frogs (*Rana sylvatica*) to both *Ribeiroia* sp. and *Telorchis* sp. These trematodes were able to penetrate the skin of *R. sylvatica* in clean and

polluted water, but a much higher number of encysted parasites were found in hosts exposed to agricultural run-off with pesticides.

There is however an alternative explanation for reported increased parasite loads in pesticide-exposed amphibians. Taylor *et al.* (2004) suggested that the cause may be a behavioural change induced by pesticides rather than an immunological change. They found that pesticides produced a reduction in tadpole swimming activity, which facilitated the penetration of echinostomatid cercariae (Bridges, 1999; Bridges and Semlitsch, 2000).

Parasites are natural stressors. In nature, animals are exposed to them along with any anthropogenic stressors that may be present. It is worth noting that contaminants and parasites occurring together in the environment may exacerbate the pathogenic effects each have on the host separately (e.g. Khan and Thulin, 1991; Lafferty and Kuris, 1999). In fact, Lafferty and Holt (2003) predicted, using theoretical models, that while the impacts of host-specific parasites are reduced in stressful conditions, those of generalist parasites are intensified.

Studies have demonstrated that when freshwater fishes are exposed to polluted conditions, parasitism can reduce their survival (Pascoe and Cram, 1977; Boyce and Yamada, 1977). However, sublethal effects in fish from the combination of parasites and pollution have also been investigated. For example, Marcogliese *et al.* (2005) found that perch (*Perca flavescens*) naturally infected with the digenetic trematode *Apophallus brevis* and the nematode *Raphidascaris acus* exhibited greater oxidative stress (as measured by lipid peroxidation in the liver) in polluted conditions compared to infected fish in clean water and uninfected fish in polluted water. The combined sublethal effects

of agricultural contaminants with other stressors have been examined in amphibians (Boone and James, 2003; Hatch and Blaustein, 2003; Relyea, 2004a, b; Relyea *et al.,* 2005). However, few studies have examined the combined pathogenic effects of parasites infecting amphibians exposed to agricultural contaminants (Kiesecker, 2002; Christin *et al.,* 2003). Further work in this area is needed to assess the realistic contributions of agriculture and parasites to amphibian declines.

Parasites as indicators of environmental disturbance

Amphibians are heavily dependent upon water and wetland habitat for reproduction and development, and thus are particularly vulnerable to aquatic contaminants, habitat fragmentation, and human activity in the surrounding area. Amphibians are undergoing alarming skeletal and limb malformations, population declines, range reductions, and even local extinctions (Phillips, 1990; Johnson *et al.,* 1999, 2001; Kiesecker *et al.*, 2001). Environmental toxicants (e.g. trace metals and industrial chemicals, etc.), climate change, UV radiation, introduction of non-native predators or competitors, fungal pathogens, habitat destruction and habitat fragmentation have all been suggested as potential causes of this decline (see review by Carey *et al.,* 2003), but recently attention has focused on pesticides (Blaustein and Wake, 1990; Carey and Bryant, 1995; Carey *et al.*, 2003), opportunisitic pathogens (Carey *et al.,* 2003), and parasites (e.g. Sessions *et al.,* 1999). Because amphibians are considered to be sentinel organisms, the problems these animals are experiencing are thought to be strongly indicative of habitat deterioration. Potentially, the parasites of amphibians may prove more sensitive as warning signals of environmental degradation due to their requirements for transmission, survival, and proliferation.

Parasites are generally recognised as good indicators of environmental disturbance (Poulin, 1992; Lafferty, 1997; Marcogliese and Cone, 1997a, b; MacKenzie, 1999; Marcogliese, 2005). The use of parasite communities to monitor environmental pollution and "ecosystem health" (Marcogliese, 2005) is an exploratory area of research, and the use of living organisms in general as bioindicators is considered by many to be coming of age (Whitfield, 2001). Invertebrate species have proven important in toxicity studies (Kevan, 1999; Maltby, 1999; Paoletti, 1999; Clements *et al.,* 2000; Le Bris and Pouliquen, 2004) as have vertebrates, particularly with regard to the biomarkers discussed above. The parasites of marine and freshwater fishes (e.g. Khan and Thulin, 1991; Poulin, 1992; Overstreet, 1993; MacKenzie *et al.*, 1995) have been used extensively as indicators of environmental pollution at both the population and community levels, and more recently the parasites of snails have been examined in the context of landscape disturbance (Kuris and Lafferty, 1994; Huspeni and Lafferty, 2004). The relationship of the parasite communities of amphibians to habitat deterioration and disturbance has never been examined.

Field studies investigating the use of parasites as indicators of ecosystem disturbance are approached in many ways. Some involve quantifying parasitism in hosts between control and polluted localities (e.g. Moser and Cowan, 1991; Koprivnikar *et al.,* 2006a) or in the same localities over a period of habitat rehabilitation (e.g. Cone *et al.,* 1993; Valtonen *et al.,* 2003; Huspeni and Lafferty, 2004). Less common are studies comparing parasitism at different distances from a point source (e.g. Siddall *et al.,* 1994; Yeomans *et al.,* 1997; Marcogliese *et al.,* 2006) or along a gradient of impact (e.g. Marcogliese and Cone, 1996, 1997b).

Impacts of environmental perturbation can be examined at many scales (Marcogliese, 2005). Studies focussing on parasites at the population level permit investigation of the responses of particular host species and parasite species to an environmental disturbance (e.g. Marcogliese *et al.,* 1990; Johnson and Chase, 2004). However, changes in parasite abundance, intensity, and/or prevalence are not necessarily consistent among species. They can vary depending on the type of disturbance and its intensity, the life cycle of the parasite, and/or how the hosts and parasites themselves are individually impacted (Poulin, 1992; Lafferty, 1997). The effects of environmental disturbance on all parasite species together are integrated at the community level. Species richness and diversity, among other measures used to quantitatively describe communities, generally decrease in disrupted habitats (Marcogliese, 2004, 2005; Lafferty, 1997) and have been demonstrated to mirror the species richness and diversity of host communities in the habitat (Hechinger and Lafferty, 2005; Hudson *et al.,* 2006). Nevertheless, these measures can be less revealing when changes to parasite community structure are not statistically significant or masked. This may be the case in more moderately disturbed and polluted habitats (Marcogliese *et al.,* 2006) or when parasites with a variety of life-histories respond differentially to environmental perturbation. Examining parasite species based on life-history type (Esch, 1971; Landsberg *et al.,* 1998; Diamant *et al.,* 1999) or shared intermediate hosts (Marcogliese and Cone, 1996, 1997b; Huspeni and Lafferty, 2004), among other types of groupings, may therefore be more informative (Marcogliese, 2005).

Concluding remarks

The ecology of parasite communities alone has intrinsic value. However, the unique position of parasites in the ecosystem also permits examination of the broader impacts of anthropogenic disturbance. Despite the warnings in Rachel Carson's *Silent Spring* in 1962, research examining the ecological implications of agricultural chemicals and land use has only recently accelerated. Agricultural activities can cause physico-chemical changes in aquatic ecosystems, which may have consequences for all organisms within and surrounding wetland habitats, including parasites. Most recent studies examine the consequences of agricultural contaminants on amphibian-parasite interactions solely within laboratory conditions (e.g. Christin *et al.,* 2003; Gendron *et al.,* 2003; Koprivnikar *et al.,* 2006b), focus on one species of parasite (Kiesecker, 2002; Gendron *et al.,* 2003; Johnson and Chase, 2004), expose the hosts and/or parasites to only one pollutant (e.g. Storrs and Kiesecker, 2004; Koprivnikar *et al.,* 2006b, respectively), or sometimes do not consider the biology of parasites and pathogens in the environmental significance of the study (Carey and Bryant, 1995; Carey *et al.,* 1999; Kiesecker, 2002; Parris and Baud, 2004). Essentially, our knowledge is incomplete, and there remains a need for studies that investigate the consequences of agriculture on the suite of parasite species in amphibians inhabiting impacted wetland habitats.

INTRODUCTION

Numerous studies have examined parasite communities in aquatic organisms exposed to disturbed environmental conditions (Poulin, 1992; Lafferty, 1997; Marcogliese and Cone, 1997b; Marcogliese, 2005). Mounting experimental and field evidence demonstrates that a variety of types of anthropogenic disturbance (e.g. urban effluents, thermal pollution, heavy metal pollution, land development) can influence the structure of parasite communities in fishes and snails (Poulin, 1992; Khan and Thulin, 1991; Lafferty, 1997; MacKenzie, 1999; Lafferty and Kuris, 2005). However, the parasite communities of amphibians have never been examined in this context. Populations of frogs, considered to be sentinel organisms of ecological problems, are in global decline (e.g. Phillips, 1990; Kiesecker *et al.,* 2001; Stuart *et al.,* 2004; Green, 2005). It is possible that the parasites of these amphibians are equally informative, or even more so, of an anthropogenically-disrupted environment.

Environmental disturbance is pervasive and, depending on the specific type and intensity, the transmission of parasites can be either impeded or enhanced. Species richness and diversity of parasite communities have been empirically shown to reflect those of their host communities (Hechinger and Lafferty, 2005). Any physico-chemical changes preventing hosts from occupying and using a habitat will influence the transmission and establishment of parasites, particularly those that depend on trophic pathways and food web structure (Cone *et al.,* 1993; Marcogliese, 2003, 2004). Therefore, changes in the intermediate and definitive host communities as a result of habitat deterioration may be inferred from the structure of parasite communities (Esch,

1971; Marcogliese and Cone, 1997b; Huspeni and Lafferty, 2004; Lafferty and Kuris, 2005).

An increasing number of freshwater ecosystems are located either near or within agricultural lands and receive run-off with pesticides and fertilisers (Lemieux *et al.,* 1995; Matson *et al.,* 1997; Vitousek *et al.,* 1997; Wang *et al.,* 1997; Carpenter *et al.,* 1998). Little is known of the impacts of agricultural activity on the amphibians that inhabit these ecosystems and the parasites that infect them. However, aquatic inputs associated with agriculture have been shown to increase the susceptibility of frogs to parasitic infection through immunosuppression (Carey and Bryant, 1995; Kiesecker, 2002; Carey *et al.,* 2003; Christin *et al.,* 2003, 2004), facilitate the transmission of parasites through intermediate host availability (Johnson and Chase, 2004), increase the establishment of parasites (Gendron *et al.,* 2003), and reduce the survival and infectivity of free-living larval stages (Pietrock and Marcogliese, 2003; Koprivnikar *et al.,* 2006b). The landscape surrounding a wetland may also reduce habitat use by definitive hosts, such as small carnivorous mammals, birds, and other amphibians, and consequently limit their respective contributions to the infective pool (Kuris and Lafferty, 1994; Huspeni and Lafferty, 2004; Hechinger and Lafferty, 2005; Koprivnikar *et al.,* 2006a).

A sudden change in the natural environment of parasites and their hosts can have sweeping consequences (Scott, 1988; Lafferty and Kuris, 1999). For example, limb and skeletal malformations, and world-wide declines of amphibian populations are attributed to mounting agricultural activity (Ouellet *et al.,* 1997; Kiesecker, 2002; Carey *et al.,* 2003; Johnson & Chase, 2004; Brynn *et al.,* 2005). Johnson and Chase (2004) linked increased eutrophication in freshwater ponds, due to agricultural run-off, to a shift in the

snail community composition towards higher densities of *Planorbella* sp., the first intermediate host of *Ribeiroia ondatrae.* This digenean parasite is known to cause developmental limb malformations in amphibians (Johnson *et al.,* 1999), and therefore anthropogenic activity causing eutrophication may be facilitating this alleged disease epidemic (Johnson and Chase, 2004; Johnson *et al.,* 2001). By experimentally exposing wood frogs (*Rana sylvatica*) to commonly used pesticides, Kiesecker (2002) was able to demonstrate an increase in the prevalence of both *R. ondatrae* and *Telorchis* sp. as a result of pesticide-induced immunosuppression. In complementary field experiments, Kiesecker (2002) found that malformations due to *R. ondatrae* were more common in wetlands receiving agricultural run-off with pesticides.

While studies focussing on particular species or groups of parasites accumulate, there continues to be an absence of studies of the parasite communities of amphibians in an environmental context. Agricultural contaminants and anthropogenically modified landscapes will impact the biodiversity of free-living invertebrate and vertebrate communities (McLaughlin and Mineau, 1995; Wang *et al.,* 1997; Tillman, 1999; Jenkins, 2003). Presumably, this will affect their parasite communities as well.

In this study, the parasite communities of leopard frogs (*Rana pipiens*) were examined in detail in wetlands exposed to varying degrees of agricultural impact in the St. Lawrence River basin, south and east of Montreal in Quebec, Canada. In order to elucidate the relationships between environmental disturbance and parasite community structure, populations and groupings of parasite species, as well as their communities, were analysed in relation to local measurements of water quality and landscape attributes potentially alterable by agriculture.

MATERIALS AND METHODS

Study localities

In the St. Lawrence River drainage basin in Quebec, nine wetlands were selected for study (Fig. 1). Étang John-Sauro (EJS), Parc Le Rocher (PLR), Île de la Commune (ICO) Ruisseau Fairbanks (RFB), Baie St. Francois (BSF), Rivière St. Francois (RSF), and Rivière Chibouet (RCH) were studied in 2004, and two more wetlands, Lac Boivin (LBO) and Île Nid d'Aigle (INA), were added in 2005. Five wetlands (EJS, PLR, LBO, INA, and ICO) were considered reference localities. Four wetlands (RFB, RSF, BSF, and RCH) received run-off from agricultural fields that were dedicated to corn production. With the exception of PLR which is an artificial wetland within a managed park and RFB which is a drainage canal, the localities were all naturally formed. Selection of these wetlands was based on long-term data on waterborne pesticides in rivers and water bodies in southern Quebec (Berryman and Giroux 1994; Giroux *et al.,* 1997) or on continuing studies related to pesticide contamination and frogs. All localities were known leopard frog breeding habitat.

Collection of environmental variables

Water samples for pesticide and nutrient analyses were collected from May to July 2005. The timing was chosen to permit detection of peak pesticide concentrations during the period of tadpole development. Repeated sampling was necessary because pesticide and nutrient concentrations can vary over time, due to a variety of environmental factors, such as rain or irrigation intensity (Brisbin and Runka, 1995; Lemieux *et al.*, 1995).

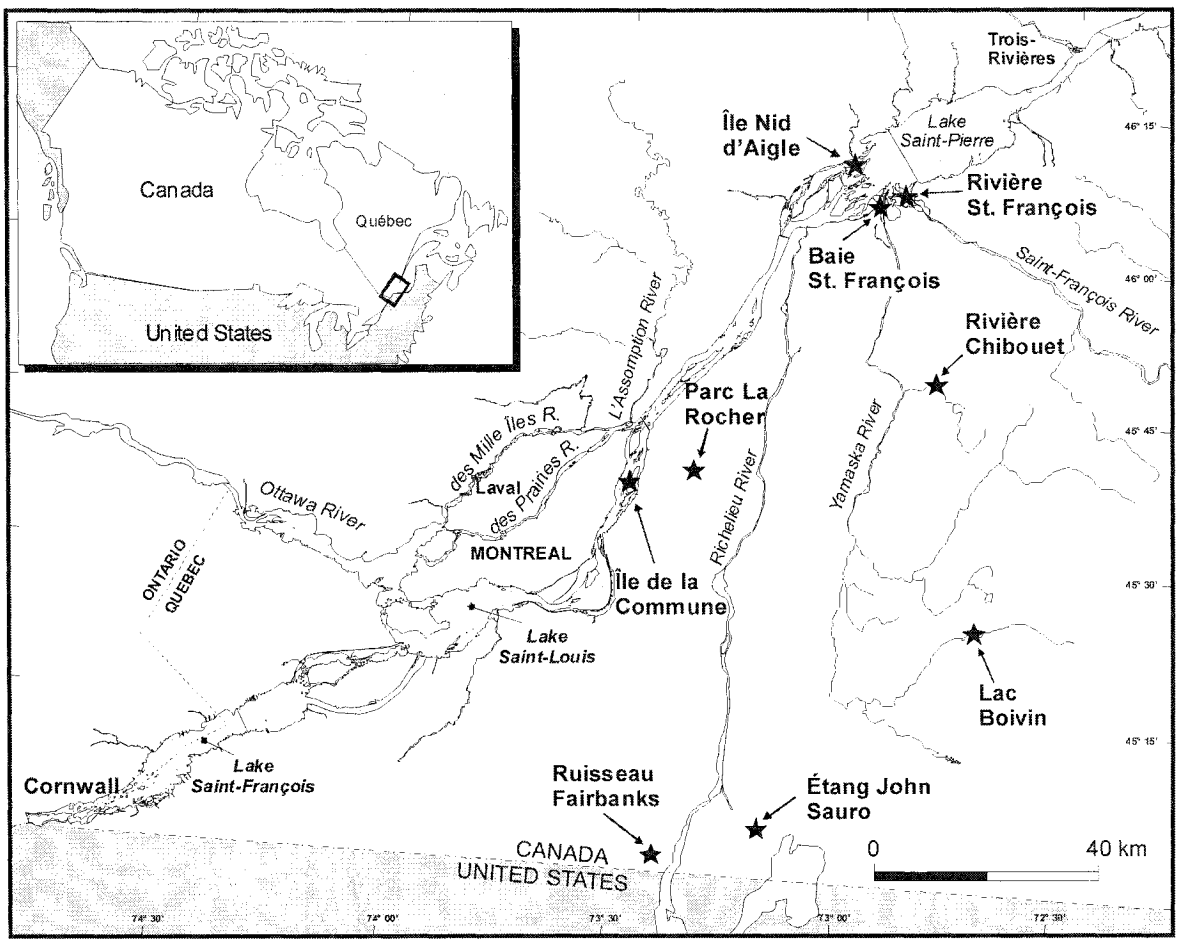

Fig. 1 - Map of study localities in the St. Lawrence River basin in Quebec, Canada.

For pesticide analysis, water samples from ICO and RFB were collected every week between 23 May and 4 July and from PLR and EJS every other week in that same time period. Water samples from INA were collected once on 29 June, from BSF and RSF every week between 26 May and 13 July, from LBO on 8 and 28 July, and from RCH every other day between 17 May and 17 July. The water samples were prepared for organophosphorous and triazine herbicide analyses (Carrier, 2001). Water samples from BSF, RSF, and RCH were analysed by the Québec Ministère du Développement durable de l'Environnement et des Parcs; the others were analysed at the National Laboratory for Environmental Testing (NLET) in Burlington, Ontario.

Water samples for nutrient analysis were collected from ICO, BSF, RCH, PLR, RFB, and EJS at biweekly intervals from 23 May to 4 July. Samples from INA were collected once at the end of 29 June and 26 July, every week from LBO between 19 May to 28 July, and from RSF once at the end of June. The water samples were prepared according to the Laboratoire du Centre Saint-Laurent (1994) protocol for the various nutrients (total and dissolved phosphorous, nitrates, nitrites, and dissolved organic carbon). Nutrients from RSF were analysed by Québec Ministère du Développement durable de l'Environnement et des Parcs, and nutrients from the other localities were analysed by NLET.

Selected habitat characteristics for all localities were recorded in July/August 2004 and 2005 prior to and during frog collection. The surface water temperatures, surface pH, and conductivity were recorded using a digital meter (YSI Model 63, Yellow Springs, Ohio, USA). Depth was measured at the approximate centre of the wetland. GPS measurements were taken at points along the perimeter of the collection locality to permit

the calculation of wetland surface area. In addition, the geographic location of the wetland, the extent of human activity in the surrounding area, the nature of the substrate, the dominant aquatic vegetation, and the animals (mammals, birds, reptiles, amphibians, fishes, invertebrates) in the vicinity were all noted during each visit (Appendix 1).

Snail density and ranid tadpole density were determined at each locality to account for productivity and host density. Surveys were conducted in mid-June 2005 when snail and tadpoles were expected to overlap temporally. Pipe samplers (Skelly *et al.,* 2003) and dip nets were used in the collections.

The pipe sampler technique employed bottomless plastic garbage cans that covered an area of 0.1017 m^2 on the bottom of the wetland. Each wetland was stratified according to major habitat type – emerging plant habitat, submergent plant habitat, and floating leaf habitat – and depth range – 0-20 cm, 21-40 cm, and 41-60 cm, when possible. Based on randomly selected numbers which firstly dictated direction (between 1 and 4) and then secondly the number of steps in that direction (between 1 and 15), the pipe sampler was positioned within a stratum in the wetland. The combination of habitat types within the sampler was then recorded and the depth was measured with a metre stick. An average of 23 (15-34) samples was made at each locality and each sample was placed at least 2 metres apart from the previous one (D. Skelly, personal communication). The entire water column inside the sampler was swept using dip nets (mesh size < 2mm). One sweep was defined as a single movement of the net from the bottom to the surface of the water within the sampler. Ten sweeps were considered sufficient to census both tadpole and snail populations within the sampler (Heyer *et al.,* 1994). In each sweep, the number of live snails, fingernail clams, and tadpoles were recorded. Young-of-the-year

snails were not counted as they were too young to harbour trematode infections transmissible to tadpoles. Snails and tadpoles were identified to family (Clarke, 1981; Desroches and Rodrigue, 2004, respectively). Dip nets were also used for sampling along the margin. The sweep length was standardised at one metre from a haphazardly selected point in the water. Habitat characteristics, depth, and the number of each family of tadpole and snail were recorded in each sweep. An average of 24 dip net sweeps (16-31) was taken at each wetland.

Landscape variables (forest area, urban area, agricultural area, and road area on a 100 and 500 m scale) were extracted using ArcGIS (ESRI) from satellite imagery.

Host and parasite collections

The term metamorph is used to describe frogs that are predispersal and have just recently emerged from their natal pond (Kendell, 2002). Between 19 and 32 metamorph leopard frogs were collected from the localities between 26 July and 6 August in 2004 and 22 July and 5 August in 2005 (Appendix 2). These collection periods permitted valid comparisons of parasite communities within and between years (Janovy *et al.*, 1992).

Leopard frogs were caught using dip nets. Only metamorphs with a snout-vent length $\leq$ 45 mm were kept (Seburn and Seburn, 1998). Frogs were killed immediately in 0.8% tricaine methane sulfonate (MS222) and subsequently stored at -80°C until examined. If the age status of the frog was in question, age was determined from the frog's longest toe phalanges (Castanet and Smirina, 1990; Smirina, 1994).

Frogs were thawed, weighed, measured from snout to vent, and sexed. Dissection followed a standard necropsy protocol, and standard parasite preservation and mounting techniques were employed (Goater and Goater, 2001). As frozen parasite specimens are

difficult to identify, fresh specimens were collected to provide a reference collection for identification purposes. Larval and adult parasites, both fresh and frozen, were identified to generic level and, if possible, to species level. Identifications were based on parasite descriptions in the literature, survey papers (e.g. Rau *et al.,* 1978; McAlpine, 1997; McAlpine and Burt, 1998; Gillilland and Muzzall, 1999; Muzzall, 2005) and monographs (Dubois, 1968, 1970; Schell, 1970; Yamaguti, 1971, 1975; Prudhoe and Bray, 1982). Once identified, all helminths in each host were sorted by species and counted.

Terminology

Quantitative descriptors were used in accordance with definitions provided in Margolis *et al.* (1982) and Bush *et al.* (1997). Prevalence is the percentage of hosts infected with a particular parasite species. Abundance is the number of individuals of a particular parasite species in a single host regardless of whether that host is infected or not; mean abundance is therefore the average number of a particular species of parasite among all members in a sample of hosts. Species richness is the total number of parasite species in a sample. Habitat descriptors are defined according to Bush *et al.* (1997). Site is used in reference to the organ or local environment of parasitic infection in the host, while locality refers to the geographic locale or external/abiotic environment where the parasite is found.

The infrapopulation comprises all individuals of a species in an individual host at a particular time. All infrapopulations consisting of parasites of a particular life-history phase at a particular time and place are referred to as component populations. The infracommunity is defined as a community of parasite infrapopulations in a single host. A

component community refers to all infrapopulations of parasites associated within a sample of a host species at a particular time (Bush *et al.,* 1997).

Data analyses

The parasite community structure at the various localities was examined at both the component and infracommunity levels using SPSS 13.0 and GraphPad Prism V4.0. Unless otherwise stated, all analyses were conducted on data from 2004 and 2005 separately. Prevalence and mean abundance of infection were calculated. Prevalences were compared by means of a chi-squared test of independence, using JMPIN 6.0. F_{max} tests were used to determine if the data were homoscedastic. Initial inspection and testing with Shapiro-Wilks revealed that the data were not normally distributed. The data could not be normalised using transformations and therefore, non-parametric analyses were used on raw data or parametric analyses were performed on ranked data (Conover and Iman, 1981). For all analyses, the critical level of significance was set at $p < 0.05$.

Snail density at each locality was determined by extrapolating the number of snails obtained from each pipe sample of area 0.1017 m^2 to the number per 1 m^2 of the wetland. Snail abundances were compared among localities by means of a Kruskal-Wallis test, followed by a Dunn's multiple comparison test to detect where the differences occurred.

Parasite data were screened to identify any inherent sampling biases. Changes in parasite abundance can result from temporal effects (Kennedy, 1993), differences between host sexes (Zuk and McKean, 1996), or differences in host size (Kuris *et al.*, 1980). A series of Mann-Whitney U tests revealed significant differences in parasite abundances within localities from 2004 to 2005 so years were treated separately in

subsequent analyses (Appendix 3). Mann-Whitney U tests were also used to test for an effect of host sex on total parasite numbers, species abundances, and infracommunity species richness. The snout-vent lengths of leopard frogs were compared among localities using a Kruskal-Wallis test. Both length and mass differed among localities despite all frogs being approximately the same age. This was not unexpected considering the plasticity of size characteristics known to occur as a result of a variety of environmental factors such as predation pressure (Laurila *et al.,* 2002), competition (Scott, 1990), length of photoperiod (Laurila *et al.*, 2001), pesticides (Relyea, 2004a), and a variety of other influences (e.g. Semlitsch *et al.,* 1988). Therefore, host size had to be taken into consideration. If snout-vent length and parasite species abundance were correlated at any locality using a Spearman-rank correlation test, the abundances at all localities were standardised for host size. To do this, the residuals from the linear regression of parasite species abundance and frog snout-vent length were obtained for each locality. In subsequent analyses comparing abundances among localities, residuals and unstandardised abundances were ranked and were used instead of actual abundance values for these parasite species. Parasite species abundances among localities were compared by means of one-way ANOVAs, and if significant differences were observed, a Tukey's *post-hoc* test followed.

MANOVAs were used to test if parasite abundances were affected by the status of the locality (agricultural verses reference). If the ranked abundances of a parasite species were found to be significantly affected with a subsequent univariate ANOVA, then differences in abundances between the two groups of localities were estimated by visual examination of abundance values.

Parasite component communities were compared among localities by grouping species by life-cycle characteristics, including infection stage, mode of infection, and life-cycle type. For comparison across localities and for each year, the proportion of the total parasite community made up of parasitic larvae or adults, parasites that infect frogs by tissue penetration or trophic transmission, and parasites that have indirect or direct life cycle patterns were calculated. Values were based on pooled infections for each locality separately in 2004 and 2005.

Component community species richness was determined for each year for each locality by summing the number of species present (Bush *et al.,* 1997). Infracommunity species richness was found by averaging the number of species present in each frog at a given locality. These latter values were subsequently compared among localities using a Kruskal-Wallis test, followed by a Dunn's multiple comparison test.

Dominance and diversity were determined for all parasite component communities, and mean diversity was calculated for all infracommunities at a given locality. Simpson's Dominance index is a heterogeneity measure weighted towards the abundances of the commonest species, thereby differentiating it from measures of species richness (Magurran, 1988). This index gives the probability that any two individuals randomly drawn from an infinitely large community will be different species (Simpson, 1949). The Shannon-Wiener Diversity index was used to compare component community diversities. This measure is appropriate for partially censused communities because it estimates the diversity of the unsampled as well as the sampled portion of the community (Magurran, 1988). It is thus applicable to the component community since the parasites in each host are a sample of the local component community, with each host being a

replicate (Bush, 1990). However, this means there will be a slight bias in the estimate because uncertainty still exists (Pielou, 1975).

Brillouin's index was used to compare infracommunity diversities. This index is used when communities are completely censused (Pielou, 1975) and thus appropriate for infracommunities where complete information on the helminth parasite fauna within an individual host can be obtained. A Kruskal-Wallis was used to test for differences in Brillouin's index values among collections, followed by a Dunn's multiple comparison test.

Beta diversity is a measure of dissimilarity among samples in terms of variety (or abundances) of species found within them (Magurran, 1988). The fewer species the various communities share, the higher the beta diversity will be. Similarity indices are used as simple measures of beta diversity and are based on pairwise comparisons. Component communities were compared with others using Jaccard's similarity index. Jaccard's index is based on presence and absence of parasite species, and each species counts equally whether rare or abundant (Magurran, 1988). The equations for all abovementioned indices can be found in Appendix 4.

For qualitative component community comparisons, the parasite data for all frogs were pooled for each locality. The matrices generated from all pair-wise comparisons of Jaccard's indices are large and unfeasible to interpret on their own. Therefore, they were summarised with cluster analyses in PHYLIP using the unweighted pair group method and arithmetic mean (UPGMA) method to give a visual interpretation of the results in the form of a dendrogram. Using this method, the clusters were joined based on the average distance between all members in the two groups (Legendre and Legendre, 1998).

Spearman-rank correlation tests were used to evaluate associations between eight environmental variables measured in 2005 (nitrates-nitrites, P_{total}, dissolved organic carbon (DOC), conductivity, wetland surface area, and forest area, urban area, and agricultural area on the 500m scale) and parasite community structure, as measured by mean parasite number, component community species richness, mean infracommunity species richness, Simpson's dominance index, Shannon-Wiener diversity index, and mean Brillouin's index. Snail density was excluded because of potential biases in the data due to heavy rainfall and a rise in water levels during collection. Landscape variables were excluded at the 100m scale because the majority of the values were zero. Road area was correlated with agricultural and urban area at the 500m scale, and so was removed from this analysis and subsequent analyses. Corrections were not required for these multiple tests because the environmental variables were considered to be predictors, not dependent variables.

Canonical correspondence analysis (CCA) was used to examine relationships among the parasite community structure and the suite of environmental variables measured (Legendre and Legendre, 1998) using CANOCO 4.0 (ter Braak and Šmilauer, 1998). The component axes resulting from CCA essentially represent gradients in species proportions, constrained by the explanatory variables (ter Braak and Šmilauer, 1998). Although the abundances of some parasite species were correlated with snout-vent length at certain localities, there were no discernable patterns (abundance of each parasite species was not correlated with frog length at the majority of localities), and so host size was not considered a covariate in the analysis. Total abundance of a parasite species was put into the species matrix. However, parasite species with an overall prevalence $\leq 2\%$

were dropped from the analysis, thereby removing the majority of the zero abundance values from the species data matrix. RSF was excluded from the analysis as many of its environmental variables were not replicated and thus not comparable with measurements from other localities collected throughout the season. In addition, dummy variables were used to represent the intensity of agricultural contamination (EJS, LBO, INA, PLR, ICO = 1; RFB, BSF = 2; RCH = 3), based on pesticide measurements.

Forward selection prior to CCA was used to identify the best set of environmental variables that significantly explained variance in the parasite data (ter Braak and Šmilauer, 1998). A detrended correspondence analysis was initially used to determine the lengths of the gradients (axes). Gradients were sufficiently long ($\geq$ 2 standard deviations) to justify use of CCA, which assumes species have a unimodal response to the environmental gradients (Jongman *et al.,* 1995). Rare taxa were down-weighted to reduce the extreme influence of rare species and of some particularly high abundance values. After the CCA was conducted with the chosen environmental variables, an unrestricted Monte-Carlo permutation test (with 999 permutations) was used to determine the significance of the canonical axes for parasite and locality variance. Bi-plots were used to better represent the species-environment and locality-environment ordinations.

RESULTS

Habitat and host characteristics

Most of the physico-chemical variables varied markedly in 2004 and 2005 (Tables 2 and 3). Although temperature varied, the measurements can differ among localities and change within localities depending on, for example, the time of day and the depth of the pond. In most localities, pH fluctuated around neutrality, but was alkaline at PLR in 2004 and at BSF and RCH in 2005. The variability among nutrient levels was comparable among localities, but overall, the concentrations of nutrients were higher at most agricultural localities. Elevated levels of nitrite-nitrates were detected at RFB and BSF and elevated levels of phosphorus (total and dissolved) were detected at ICO and BSF, and of total phosphorus at RCH. Dissolved organic carbon was low at RSF and high at ICO and BSF. In both years, conductivity was high at RCH and was higher at RFB in 2004 and ICO in 2005. A suite of over thirty pesticides was measured at each locality, but only concentrations of atrazine and metolachlor are shown due to their common occurrence. These two pesticides were found at their highest concentrations at RCH. Atrazine levels were more moderate at LBO, RFB, RSF, and BSF, and only trace amounts were detected at EJS, INA, PLR, and ICO. Except for those at RCH, levels of metolachlor were relatively consistent among reference and moderately contaminated agricultural localities, although the concentrations at BSF were sometimes twice that of the remaining localities. The wetland surface areas of RFB, RSF, and ICO were the largest and those of INA, PLR, and RCH were the smallest. On both 100m and 500m scales, RFB had the greatest forest cover, PLR was the most urbanised, PLR and RCH were the most cultivated, and RCH was surrounded by the greatest road surface area.

Table 2 – Physical and chemical characteristics of reference and agricultural localities sampled in 2004. DNC = data not collected. Agricultural localities are italicized. Reference localities: Étang John-Sauro (EJS), Lac Boivin (LBO), Île Nid d'Aigle (INA), Parc Le Rocher (PLR), and Île de la Commune (ICO). Agricultural localities: Ruisseau Fairbanks (RFB), Baie St. Francois (BSF), Rivière St. Francois (RSF), and Rivière Chibouet (RCH).

	EJS	PLR	ICO	*RFB*	*BSF*	*RSF*	*RCH*
Temperature (°C)	22.9	24.1	23.9	21.0	DNC	24.0	30.5
pH	6.72	8.50	6.72	7.07	DNC	6.72	6.73
Conductivity (µS/cm)	123.0	199.0	175.6	1 533.0	DNC	124.4	1 430.0
Surface area (m^2)	3 909	3 013	19 260	13 330	6 360	18 270	1 132

Table 3 – Physical and chemical characteristics of reference and agricultural localities sampled in 2005. Mean values presented with ranges in parentheses. DNC = data not collected; * = on 100 m/500 m scale. Agricultural localities are italicized. Abbreviations for localities are explained in Table 2.

	EJS	LBO	INA	PLR	ICO	*RFB*	*BSF*	*RSF*	*RCH*
Temperature (°C)	30.7	22.6 (22.3-22.8)	25.8 (24.3-27.3)	17.8 (12.6-20.6)	20.7 (11.4-31.1)	18.5 (12.3-25.2)	26.4	20.1	18.6 (15.2-22.0)
pH	7.30	6.80 (6.70-6.90)	7.51 (7.23-7.81)	7.48 (7.20-8.03)	7.22 (6.89-7.50)	7.34 (6.96-7.65)	8.66	7.05	8.21 (8.10-8.30)
$NO_2 - NO_3$ (mg/L)	0.04	0.73 (0.04-3.59)	0.07 (0.04-0.09)	0.04	0.04	3.27 (0.52-10.21)	1.18 (0.04-3.17)	0.04	0.24 (0.04-0.74)
P_{TOTAL} (mg/L)	0.14 (0.06-0.25)	0.06 (0.02-0.13)	0.08 (0.05-0.10)	0.03 (0.02-0.04)	0.42 (0.14-0.94)	0.07 (0.05-0.09)	0.26 (0.13-0.42)	0.01	0.23 (0.05-0.52)
$P_{DISSOLVED}$ (mg/L)	0.08 (0.05-0.14)	0.05 (0.04-0.06)	0.05 (0.03-0.07)	0.01 (0.01-0.02)	0.26 (0.09-0.71)	0.04 (0.02-0.05)	0.13 (0.03-0.42)	DNC	0.06 (0.02-0.11)
DOC (mg/L)	12.15 (8.11-15.76)	7.72 (7.60-7.93)	8.57 (4.72-12.41)	11.57 (9.73-12.67)	17.43 (6.20-27.36)	7.46 (5.98-10.06)	13.81 (8.85-27.24)	0.25	12.35 (9.85-14.79)
Conductivity (µS/cm)	175.0	168.0 (159-177)	181.6 (149-207)	309.0 (266-381)	776.5 (423-1 130)	427.3 (276-595)	337.5	170.3	643.0 (556-730)
Atrazine (µg/L)	0.02 (0.02-0.04)	0.30 (0.08-0.53)	0.08	0.02 (0.01-0.03)	0.04 (0.02-0.06)	0.19 (0.03-0.35)	0.26 (0.02-0.80)	0.05 (0.02-0.13)	0.75 (0.0-3.70)
Metolachlor (µg/L)	0.03	0.01	0.04	0.03	0.06 (0.02-0.15)	0.07 (0.03-0.14)	0.14 (0.03-0.52)	0.01 (0.01-0.03)	0.329 (0.04-0.89)
Surface area (m^2)	3 909	5 098	880	1 802	20 380	13 330	6 360	18 270	1 453
Forest* (m^2)	1 025 / 9 125	0 / 1 025	0 / 0	0 / 7 125	0 / 275	4 375 / 304 375	0 / 1 300	0 / 75	100 / 4 525
Urban* (m^2)	0 / 1 550	0 / 2 700	0 / 0	125 / 2 650	0 / 0	0 / 1 875	0 / 525	0 / 0	200 / 1 000
Agriculture* (m^2)	0 / 4 375	0 / 146 875	0 / 2 500	18 750 / 369 375	1 250 / 8 125	0 / 243 125	0 / 0	0 / 0	11 875 / 547 500
Road* (m^2)	0 / 0	0 / 39 375	0 / 0	0 / 26 875	0 / 0	0 / 15 625	625 / 10 625	0 / 0	3 125 / 37 500

The mean snout-vent lengths of metamorph leopard frogs caught in 2004 and 2005 differed significantly among localities (2004: $\chi^2 = 102.06$, $p < 0.001$, $df = 6$; 2005: $\chi^2 = 180.58$, $p < 0.001$, $df = 8$). However, there were no consistent patterns in these lengths within or between reference and agricultural localities from year to year. Snail density varied among reference and agricultural localities in June 2005 ($\chi^2 = 65.94$, $p < 0.001$, $df = 8$) (Fig. 2). Lower snail densities at LBO, INA, RFB, BSF, and RSF compared to those at EJS, PLR, ICO and RCH may have been the result of a heavy, continuous rainfall just prior to the snail collections at the first group of localities. Few consistent differences were evident between agricultural and reference localities before or after the rainfall. However, RFB, BSF, and RSF had significantly higher snail densities than those observed at INA. Too few tadpoles were collected at each locality to accurately assess tadpole density (data not shown).

Composition of parasite communities

Eighteen helminth parasite species, of which twelve were digeneans and six were nematodes, were found in 203 leopard frogs collected in 2004 (Table 4). These same species were found in 270 leopard frogs in 2005, along with two others, *Halipegus* sp. and *Proteocephalus* sp (Table 5). Most parasites were identified to at least the generic level, but three groups of larval parasites could be identified only to the family level (Echinostomatidae, Strigeidae, and Gorgoderidae) and one to the level of order (Spirurida).

Three larval digenean species and one nematode species were the most prevalent and/or abundant in the parasite communities of leopard frogs collected in this study. In

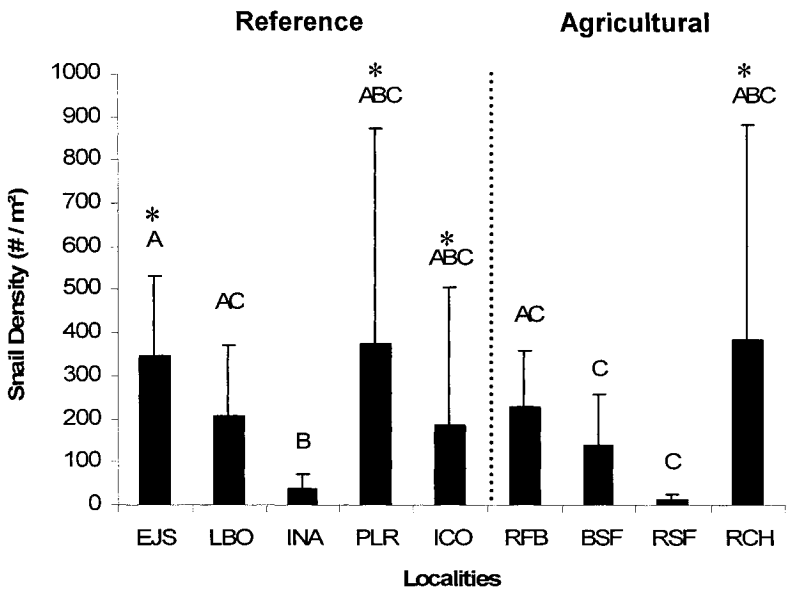

Fig. 2 – Snail density (# snails/m²) at reference and agricultural localities in June 2005. Values differed significantly according to arrangement of letters. Standard deviation bars are presented. * Localities sampled prior to heavy rainfall; other localities were sampled afterwards. Abbreviations for localities are explained in Table 2.

Table 4 – Prevalence and mean abundance (± 1 S.D.) of parasite species found in *Rana pipiens* in 2004 (n = 19-32 frogs per locality). % = prevalence. Ab = Mean Abundance. Agricultural localities are italicized. Parasite prevalence varied significantly among localities when species name is in bold. Values differed significantly within a year according to arrangement of letters. Abbreviations for localities are explained in Table 2.

	Localities													
	EJS		PLR		ICO		*RFB*		*BSF*		*RSF*		*RCH*	
	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab
Digenea														
***Alaria* sp.** (mesocercaria, post-mesocercaria; body and eye muscle, body cavity)	3.1	0.03^{B} (0.2)	0	0	16.7	0.4^{AB} (1.2)	6.5	0.1^{AB} (0.6)	0	0	13.3	0.2^{A} (0.5)	0	0
Apharyngostrigea pipientis (metacercaria tetracotyle; body cavity)	18.8	0.8 (2.9)	3.3	0.8 (4.2)	23.3	4.1 (14.9)	12.9	0.7 (2.2)	15.8	0.2 (0.4)	0	0	0	0
Clinostomum sp. (metacercaria; body and eye Muscle, brain)	15.6	0.3 (0.9)	0	0	13.3	4.0 (14.7)	0	0	0	0	0	0	0	0
***Diplostomum* spp.** (metacercaria; eye lens and humour, brain)	3.1	0.03^{B} (0.2)	10.0	0.1^{B} (0.4)	16.7	0.2^{B} (0.6)	32.3	0.5^{A} (0.9)	0	0	0	0	0	0
***Echinostoma* sp.** (metacercaria; kidney)	93.8	29.7 (36.5)	3.3	62.6 (73.5)	96.7	17.9 (19.6)	80.7	10.8 (37.2)	42.1	2.2 (4.5)	13.3	0.6 (1.9)	96.8	26.0 (53.9)
Echinostomatidae gen. sp. (metacercaria; body and eye muscle)	31.1	0.2 (1.1)	10.0	0.03 (0.2)	3.3	0.8 (4.6)	6.5	0.1 (0.3)	0	0	6.7	0.1 (0.3)	0	0

	EJS		PLR		ICO		*RFB*		*BSF*		*RSF*		*RCH*	
	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab
***Fibricola* sp.** (metacercaria; body muscle, body cavity, under skin)	34.4	12.2^{BC} (60.7)	0	0	3.3	0.2^{C} (0.9)	9.7	0.1^{C} (0.3)	0	0	43.3	20.7^{B} (71.3)	80.7	175.3^{A} (254.9)
Glypthelmins quieta (adult; intestine)	12.5	0.6 (2.7)	0	0	6.7	0.1 (0.3)	0	0	0	0	3.3	0.03 (0.2)	0	0
Gorgoderina attenuata (adult; urinary bladder)	18.8	0.2^{AB} (0.5)	0	0	13.3	0.3^{A} (1.3)	35.5	0.6^{B} (0.9)	0	0	13.3	0.1^{AB} (0.4)	0	0
Gorgoderidae gen. sp. (metacercaria; body cavity, body and eye muscle, on and under skin, surface of heart, lungs, liver, kidney)	21.3	5.6 (17.6)	0	0	93.3	58.3 (70.7)	74.2	5.6 (6.9)	21.6	4.1 (9.3)	3.3	0.1 (0.4)	6.5	1.1 (5.4)
***Haematoloechus* spp.** (immature, adult; lungs)	50.0	8.8^{A} (15.2)	3.3	0.03^{B} (0.2)	3.3	0.2^{B} (0.9)	35.5	5.4^{A} (16.8)	5.3	1.2^{B} (5.3)	3.3	0.1^{B} (0.4)	0	0
Strigeidae gen. sp. (metacercaria tetracotyle; muscle)	12.5	0.2 (0.5)	0	0	0	0	3.2	0.03 (0.2)	5.3	0.1 (0.2)	0	0	3.2	0.1 (0.5)
Nematoda														
Cosmocercoides dukae (larva, adult; intestine)	3.1	0.03 (0.2)	0	0	3.3	1.0 (5.5)	3.2	0.03 (0.2)	0	0	10.7	0.2 (0.7)	0	0
***Oswaldocruzia* sp.** (larva, adult; intestine)	0	0	3.3	0.03^{B} (0.18)	40.0	55.7^{A} (228.5)	6.5	0.2^{B} (1.0)	15.8	1.1^{AB} (3.1)	50.0	2.1^{A} (3.4)	3.3	0.03 (0.2)
Rhabdias ranae (immature, adult; body cavity, Lungs)	15.6	0.4^{B} (1.3)	0	0	63.3	2.8^{A} (6.0)	6.5	0.2^{B} (1.0)	21.1	1.1^{B} (3.1)	30.0	0.5^{B} (1.1)	3.2	0.03^{B} (0.2)

	EJS		PLR		ICO		*RFB*		*BSF*		*RSF*		*RCH*	
	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab
***Spiroxys* sp.** (larva; stomach surface, liver, body muscle)	31.3	0.4^{B} (0.6)	23.3	0.3^{AB} (0.6)	6.7	0.2^{AB} (0.8)	22.6	0.2^{AB} (0.4)	0	0	3.3	2.2^{A} (12.2)	32.3	0.03^{B} (0.2)
Spirurida gen. sp. (larva; liver)	50.0	5.7^{A} (9.2)	0	0	3.3	0.2^{C} (0.9)	90.3	4.1^{B} (3.4)	0	0	3.3	1.9^{C} (10.2)	3.2	0.03^{C} (0.2)
***Strongyloides* sp.** (adult; intestine)	3.1	31.2 (176.6)	0	0	16.7	1.5 (5.5)	22.6	33.5 (179.2)	5.3	0.3 (1.1)	23.3	0.7 (1.7)	3.2	0.03 (0.2)

Table 5 – Prevalence and mean abundance (± 1 S.D.) of parasite species found in *Rana pipiens* in 2005 (n = 30 frogs per locality). % = prevalence. Ab = Mean Abundance. Agricultural localities are italicized. Parasite prevalence varied significantly among localities when species name is in bold. Values differed significantly within a year according to arrangement of letters. Abbreviations for localities are explained in Table 2.

	Localities																	
	EJS		LBO		INA		PLR		ICO		*RFB*		*BSF*		*RSF*		*RCH*	
	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab
Digenea																		
***Alaria* sp.** (mesocercaria, post-mesocercaria; body and eye muscle, body cavity)	30.0	1.3 (2.9)	13.3	0.9 (4.6)	13.3	1.0 (4.9)	0	0	26.7	0.3 (0.5)	10.0	0.3 (1.0)	30.0	1.8 (4.7)	13.3	0.4 (1.5)	3.3	0.1 (0.7)
Apharyngostrigea pipientis (metacercaria tetracotyle; body cavity)	10.0	0.1 (0.4)	26.7	1.6 (4.1)	13.3	0.1 (0.4)	10.0	0.5 (1.6)	13.3	0.2 (0.5)	16.7	0.3 (0.8)	63.3	2.8 (4.4)	10.0	0.1 (0.3)	0	0
Clinostomum sp. (metacercaria; body and eye muscle, brain)	16.7	0.4 (1.3)	6.7	0.3 (1.5)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
***Diplostomum* spp.** (metacercaria; eye lens and humour, brain)	0	0	0	0	83.3	19.9 (23.9)	33.3	2.0 (6.9)	13.3	0.1 (0.4)	33.3	1.2 (5.5)	0	0	6.7	0.1 (0.3)	0	0
***Echinostoma* sp.** (metacercaria; kidney)	100	44.8 (53.9)	80.0	42.6 (75.0)	70.0	13.2 (28.6)	100	252.8 (206.9)	93.3	10.2 (9.3)	46.7	2.7 (4.6)	93.8	8.1 (12.1)	60.0	9.8 (33.2)	56.7	5.6 (9.9)
Echinostomatidae gen. sp. (metacercaria; body and eye muscle)	0	0	0	0	3.3	0.03 (0.2)	0	0	0	0	6.7	0.4 (1.9)	0	0	3.3	0.03 (0.2)	0	0

	EJS		LBO		INA		PLR		ICO		*RFB*		*BSF*		*RSF*		*RCH*	
	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab
***Fibricola* sp.** (metacercaria; body muscle, body cavity)	70.0	20.4^{B} (49.3)	23.3	13.5^{B} (46.0)	90.0	348.2^{A} (525.3)	6.7	0.1^{B} (0.4)	83.3	6.1^{B} (5.7)	23.3	3.5^{C} (11.3)	43.3	37.6^{B} (185.8)	100	2373.9^{A} (1353.8)	80.0	33.1^{B} (46.0)
Glypthelmins quieta (adult; intestine)	26.7	1.1^{A} (2.6)	10.0	0.2^{B} (0.6)	0	0	0	0	3.3	0.03^{B} (0.2)	6.7	0.1^{B} (0.3)	3.3	0.03^{B} (0.2)	3.3	0.1^{B} (0.4)	0	0
Gorgoderina attenuata (adult; urinary bladder)	10.0	0.1^{AB} (0.4)	3.3	0.1^{AB} (0.4)	36.7	0.6^{A} (0.9)	0	0	56.7	1.1^{AB} (1.6)	10.0	0.2^{AB} (0.6)	3.3	0.03^{AB} (0.2)	23.3	0.3^{B} (0.7)	0	0
Gorgoderidae gen. sp. (metacercaria; body cavity, body and eye muscle, on and under skin, surface of heart, lungs, liver, kidney)	86.7	43.4 (86.9)	46.7	9.4 (35.1)	40.0	0.9 (1.5)	6.7	0.4 (1.5)	100	127.7 (59.2)	50.0	1.8 (3.1)	93.3	33.5 (45.2)	33.3	0.9 (1.9)	0	0
***Haematoloechus* spp.** (immature, adult; lungs)	50.0	12.2^{A} (21.6)	3.3	0.1^{B} (0.6)	6.7	0.1^{B} (0.3)	6.7	0.2^{B} (0.6)	3.3	0.03^{B} (0.2)	3.3	0.1^{B} (0.7)	0	0	3.3	1.5^{B} (8.2)	0	0
***Halipegus* sp.** (immature; intestine)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.3	0.03 (0.2)
Strigeidae gen. sp. (metacercaria tetracotyle; muscle)	0	0	3.3	0.1 (0.4)	0	0	0	0	0	0	3.3	0.1 (0.7)	0	0	0	0	0	0
Nematoda																		
Cosmocercoides dukae (immature, adult; intestine	6.7	0.1 (0.6)	36.7	1.3 (2.7)	3.3	0.1 (0.7)	10.0	0.2 (0.8)	6.7	1.0 (4.0)	10.0	0.6 (2.6)	0	0	36.7	1.1 (3.1)	0	0

	EJS		LBO		INA		PLR		ICO		*RFB*		*BSF*		*RSF*		*RCH*	
	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab	%	Ab
***Oswaldocruzia* sp.** (adult; intestine)	6.7	0.1^{A} (0.4)	13.3	0.3^{B} (1.0)	33.3	3.9^{AB} (8.2)	3.3	0.03^{AB} (0.2)	10.0	0.1^{AB} (0.4)	6.7	0.4^{B} (1.5)	20.0	0.2^{AB} (0.5)	3.3	1.4^{AB} (4.0)	3.3	0.03^{AB} (0.2)
Rhabdias ranae (immature, adult; body cavity, lungs)	13.3	0.2 (0.6)	16.7	0.6 (1.9)	70.0	4.5 (7.1)	16.7	0.2 (0.5)	30.0	0.5 (1.0)	0	0	26.7	0.6 (1.4)	23.3	0.5 (1.0)	3.3	0.03 (0.2)
Spiroxys sp. (larva; surface of stomach, liver, body muscle)	16.7	0.2 (0.6)	3.3	0.1 (0.4)	3.3	0.03 (0.2)	0	0	6.7	0.1 (0.3)	3.3	0.03 (0.2)	10.0	0.1 (0.3)	6.7	0.1 (0.4)	0	0
Spirurida gen. sp. (larva; liver)	33.3	1.4^{AB} (2.9)	23.3	0.4^{A} (0.8)	0	0	0	0	0	0	0	0	3.3	0.03^{B} (0.2)	0	0	0	0
***Strongyloides* sp.** (adult; intestine)	13.3	1.3 (5.5)	26.7	2.3 (7.9)	16.7	0.5 (1.4)	0	0	50.0	6.4 (9.3)	0	0	10.0	0.3 (1.2)	60.0	2.6 (3.8)	3.3	0.03 (0.2)
Cestoda																		
Proteocephalus sp. (proteocephalid; intestine)	0	0	0	0	3.3	0.03 (0.2)	0	0	0	0	6.7	0.3 (1.2)	0	0	0	0	0	0

2004, *Echinostoma* sp. was the dominant species in the parasite communities of EJS, ICO, and RCH parasitizing 94-97% of frogs at those localities; *Fibricola* sp. was the most abundant in RSF and RCH; Gorgoderidae gen. sp. was the most abundant parasite species in ICO; and *Oswaldocruzia* sp. was the most prevalent in RSF. In 2005, *Echinostoma* sp. was the most prevalent in EJS, LBO, PLR, and BSF, parasitizing 80-100% of the frogs; *Fibricola* sp. was the most abundant and prevalent in INA, RSF, and RCH with infection rates of over 80%; Gorgoderidae gen. sp. was the most prevalent at ICO and RFB, and also the most abundant in the former locality. Only *Echinostoma* sp. was found at all localities in both years.

No sex-related differences were found in the infracommunity richness (2004: $U = 5105.5$, $p = 0.91$, $n_{males} = 100$, $n_{females} = 103$; 2005: $U = 8756$, $p = 0.58$, $n_{males} = 131$, $n_{females} = 139$), total helminth numbers (2004: $U = 4505.5$, $p = 0.34$; 2005: $U = 9012$, $p = 0.89$), or individual parasite species abundances (Appendix 5). Accordingly, data from both sexes were pooled for all subsequent analyses. Spearman-rank correlations between the abundances of parasite species and host length in both years revealed only 2 significant associations in 2004 and 15 associations in 2005 (Appendices 6 and 7). Where there were significant associations, nematode species were generally positively associated with snout-vent length, whereas digenean species were negatively associated with snout-vent length.

Comparisons of parasite communities among localities

MANOVAs were used to test whether the abundances of individual parasite species were influenced by the pesticide contamination status of the locality (agricultural versus reference) in 2004 and 2005 (Table 6). Both overall MANOVAs were significant

Table 6 – Univariate results from MANOVA to determine if individual parasite species abundances were affected by agricultural pesticide contamination status of the localities in 2004 and 2005. Significant values are in bold.

Parasite Species	Year	Univariate ANOVA Results		Agricultural vs. Reference
		F	p	
Alaria sp.	2004	1.240	0.267	-
	2005	**3.246**	**0.049**	R > A
Apharyngostrigea pipientis		**9.266**	**0.003**	R > A
		1.477	0.229	-
Diplostomum spp.		1.094	0.297	-
		1.239	0.270	-
Echinostoma sp.		3.611	0.059	-
		0.346	0.559	-
Echinostomatidae gen. sp.		0.005	0.942	-
		0.966	0.330	-
Fibricola sp.		**8.181**	**0.005**	A > R
		81.598	**< 0.001**	A > R
Glypthelmins quieta		**8.365**	**0.005**	R > A
		< 0.001	0.996	-
Gorgoderina attenuata		0.002	0.962	-
		1.535	0.220	-
Gorgoderidae gen. sp		3.688	0.057	-
		0.226	0.636	-
Haematoloechus spp.		**7.530**	**0.007**	R > A
		0.003	0.956	-
Strigeidae gen. sp.		1.469	0.227	-
		-	-	-
Cosmocercoides dukae		0.019	0.890	-
		1.273	0.264	-
Oswaldocruzia sp.		0.060	0.807	-
		1.026	0.315	-
Rhabdias ranae		**13.653**	**< 0.001**	R > A
		0.018	0.893	-
Spiroxys sp.		**5.282**	**0.023**	A > R
		0.329	0.569	-
Spirurida gen. sp.		0.295	0.588	-
		-	-	-
Strongyloides sp.		0.222	0.638	-
		0.110	0.741	-

(2004: Wilks $\lambda = 0.48$, $p < 0.001$; 2005: Wilks $\lambda = 0.27$, $p < 0.001$). In 2004, *Fibricola* sp. and *Spiroxys* sp. were more abundant in the agricultural localities, while *A. pipientis, G. quieta, Haematoloechus* spp., and *R. ranae* were more abundant in the reference localities. In 2005, *Fibricola* sp. was more abundant in the agricultural localities and *Alaria* sp. in the reference localities. Only the abundances of *Fibricola* sp. were consistently influenced by the pesticide contamination status of the locality.

Several patterns emerged when parasite component communities were subdivided according to life-history stage (larva or adult), mode of infection (penetration or trophic transmission), and life-cycle type (indirect or direct) in 2004 and 2005. The parasite communities at all localities were dominated, in terms of relative abundance, by larval stages, parasites that infect by cutaneous penetration and by those that have indirect life-cycles (Figs. 3–5). However, the proportional representation of each group differed among localities. Frogs at PLR and RCH had the fewest adults and the largest proportions of larval parasites, while EJS, ICO, BSF, and RFB had the highest proportions of adults (Fig. 3). The frogs from EJS and RFB consistently had the highest proportions of parasites acquired through the food chain, while frogs from LBO, INA, PLR, ICO, and RCH were consistently infected with relatively more tissue penetrating parasites (e.g. cercariae and nematode larvae) (Fig. 4). The adult parasites in frogs from LBO, INA, RFB, ICO, and BSF were mostly direct-life cycle parasites that penetrate the skin, such as *C. dukae, R. ranae, Oswaldocruzia* sp., and *Strongyloides* sp. These nematodes were rarely, if ever, encountered in frogs from PLR and RCH. Frogs from these two localities consistently had the highest relative abundance of larval digeneans. A

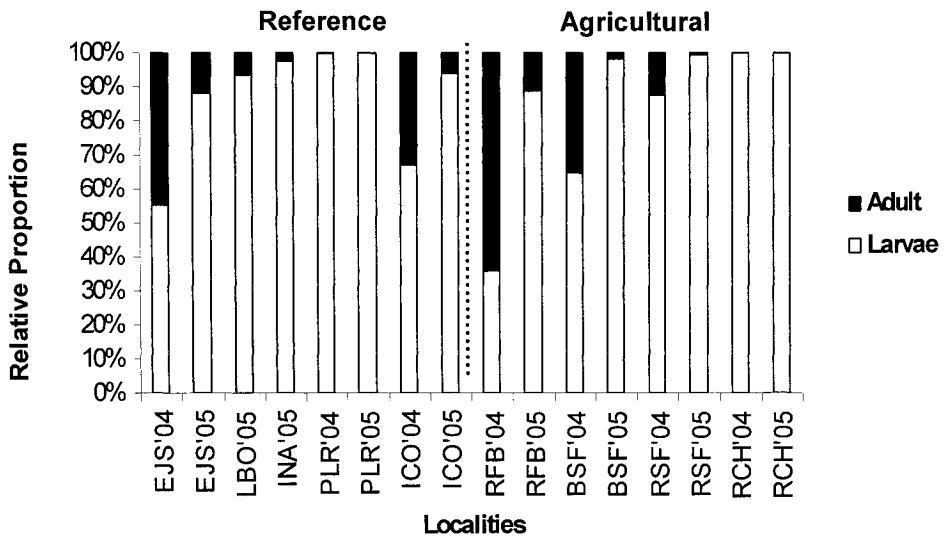

Fig. 3 – Relative proportions of parasite component communities in *Rana pipiens* grouped by life-history stage at reference and agricultural localities sampled in July/August 2004 and 2005. Values represent the proportional abundance of the total parasite community using the frogs as intermediate hosts (larvae) or definitive hosts (adults). Abbreviations for localities are explained in Table 2.

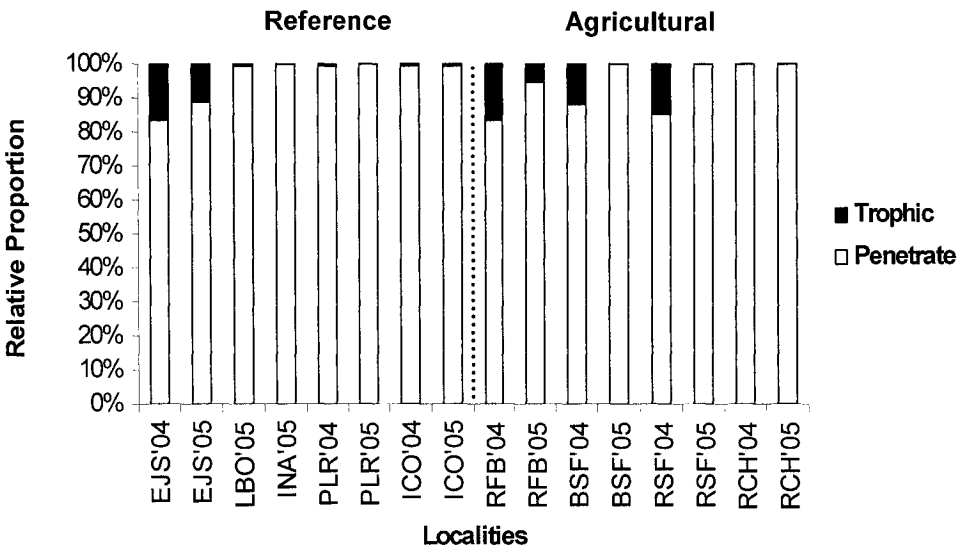

Fig. 4 – Relative proportions of parasite component communities in *Rana pipiens* grouped by transmission mode at reference and agricultural localities sampled in July/August 2004 and 2005. Values represent the proportional abundance of the total parasite community infecting frogs by penetration and by trophic transmission. Abbreviations for localities are explained in Table 2.

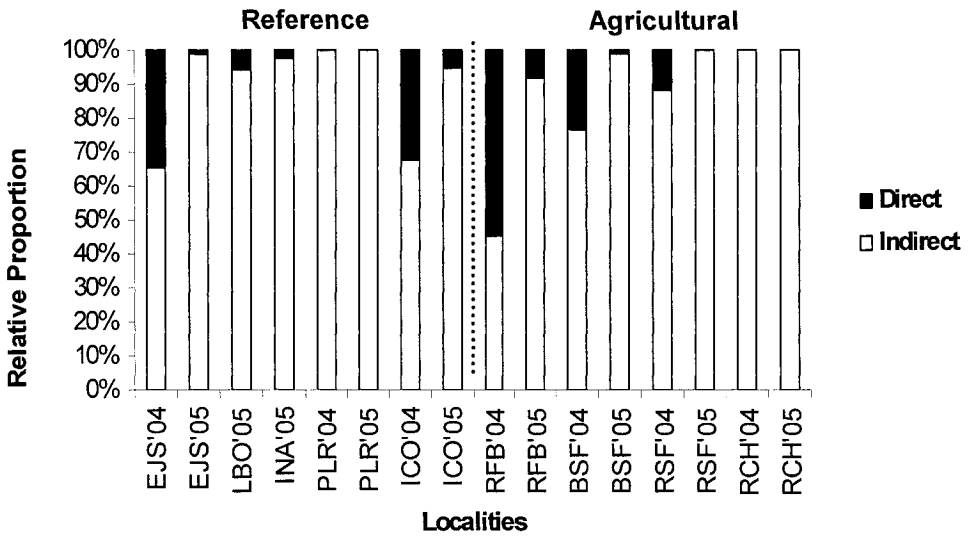

Fig. 5 – Relative proportions of parasite component communities in *Rana pipiens* grouped by life-cycle type at reference and agricultural localities sampled in July/August 2004 and 2005. Values represent the proportional abundance of the total parasite community having either a direct life-cycle (one host) or indirect life-cycle (more than one host). Abbreviations for localities are explained in Table 2.

large relative abundance of adult parasites at EJS, RFB, and RSF are acquired through trophic pathways, including *Haematoloechus* spp., and *G. attenuata*. A temporal pattern was evident as well. From 2004 to 2005, there was a decrease in the relative proportion of adults, parasites requiring trophic transmission, and parasites with direct life-cycles at all localities except of RCH and PLR where the relative abundances of these parasites remained consistently low.

Species richness was determined for both the component community and the infracommunities at each locality. The component communities at PLR and RCH were consistently among the most depauperate, and there was little difference among the rest of the localities (Fig. 6). Component community species richness at BSF was comparable to PLR and RCH in 2004. Differences in infracommunity species richness varied significantly among localities (2004: $\chi^2 = 102.25$, $p < 0.001$, $df = 6$; 2005: $\chi^2 = 134.35$, $p < 0.001$, $df = 8$) (Fig. 7). In 2004, frogs at EJS, RFB, and ICO had among the most species rich infracommunities, while those from BSF, RSF, PLR, and RCH had the least number of species. In 2005, species richness in frogs from PLR and RCH were significantly lower than those from all other localities.

The Simpson's Dominance Index (Fig. 8) and Shannon-Wiener index (Fig. 9) revealed that the parasite component communities at INA, PLR, RSF, and RCH had the greatest dominance and the lowest diversity. A lower degree of dominance and higher diversity were encountered at EJS, RFB, and BSF in both years, in ICO in 2004, and in LBO in 2005. Brillouin's diversity index for parasite infracommunities (Fig. 10) corroborated the general pattern seen at the component community level. Significant

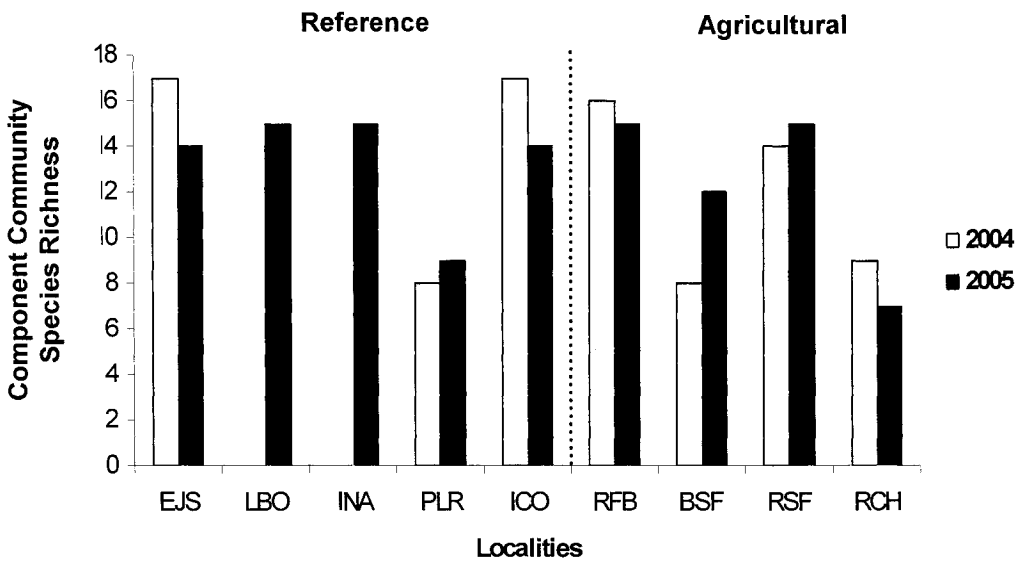

Fig. 6 – Parasite component community species richness of *Rana pipiens* at reference and agricultural localities sampled in July/August 2004 and 2005. Abbreviations for localities are explained in Table 2.

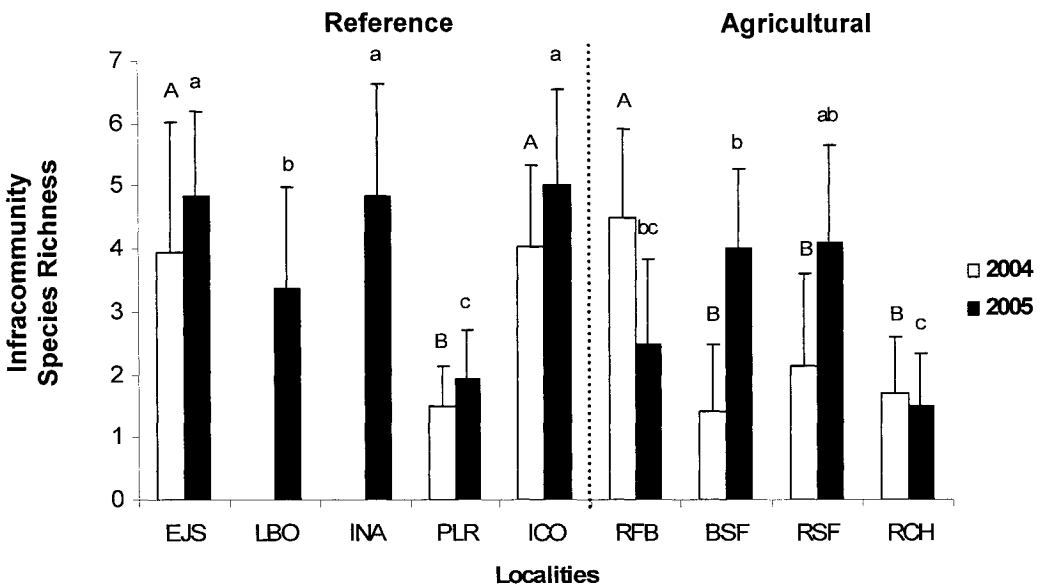

Fig. 7 – Mean parasite infracommunity species richness of *Rana pipiens* at reference and agricultural localities sampled in July/August 2004 and 2005. Values differed significantly within a year according to arrangement of letters (upper case: 2004, lower case: 2005). Standard deviation bars are presented. Abbreviations for localities are explained in Table 2.

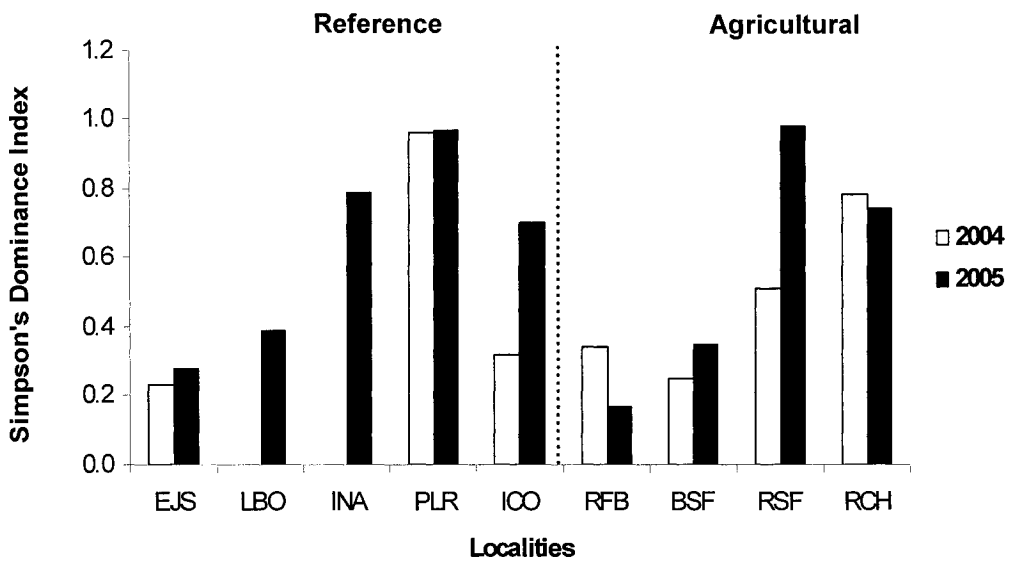

Fig. 8 – Simpson's Dominance Index for parasite component communities of *Rana pipiens* at reference and agricultural localities sampled in 2004 and 2005. Abbreviations for localities are explained in Table 2.

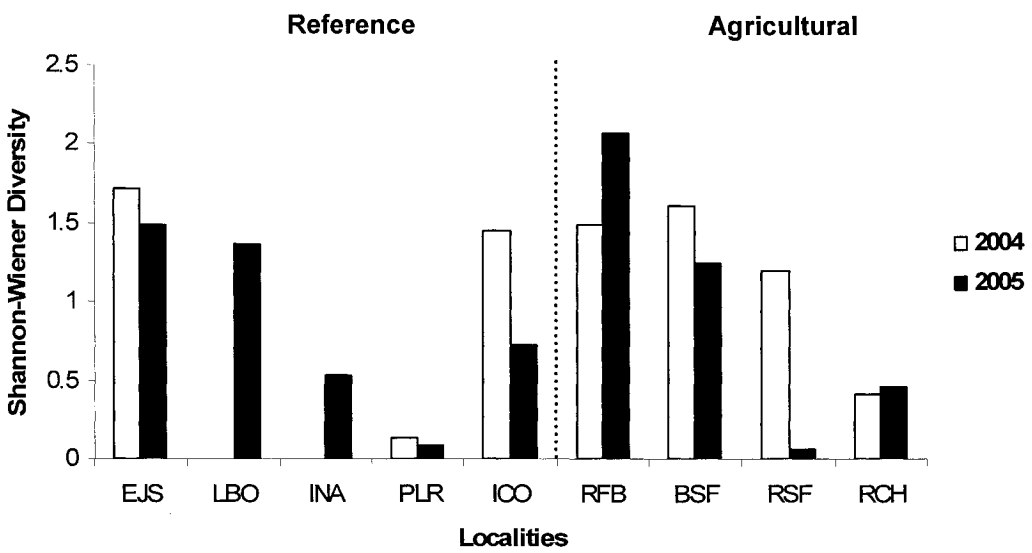

Fig. 9 – Shannon-Wiener Diversity Index for parasite component communities of *Rana pipiens* at reference and agricultural localities sampled in 2004 and 2005. Abbreviations for localities are explained in Table 2.

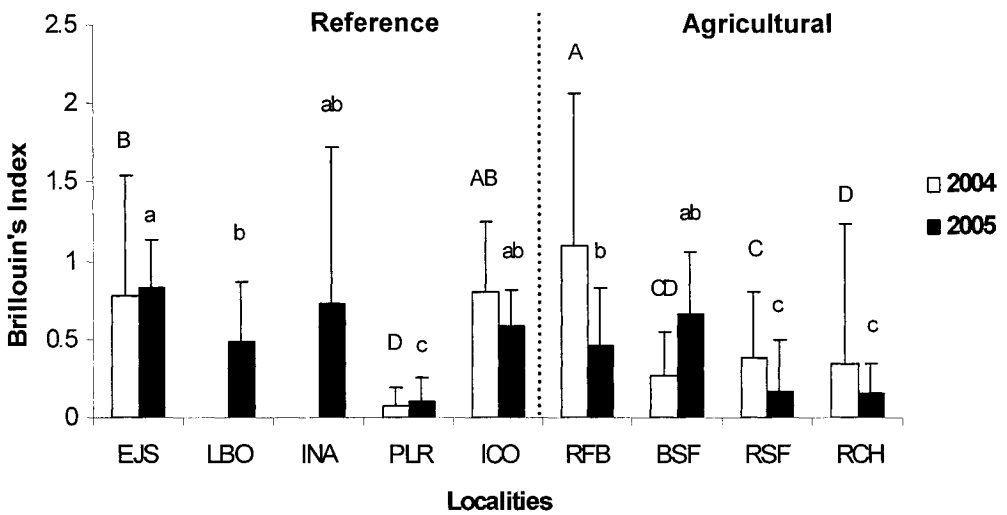

Fig. 10 – Mean Brillouin's Diversity Index for parasite infracommunities of *Rana pipiens* at reference and agricultural localities sampled in 2004 and 2005. Values differed significantly within a year according to arrangement of letters (upper case: 2004, lower case: 2005). Standard deviation bars are presented. Abbreviations for localities are explained in Table 2.

differences were observed among localities in 2004 and 2005 (2004: $\chi^2 = 91.93$, $p < 0.001$, $df = 6$; 2005: $\chi^2 = 107.37$, $p < 0.001$, $df = 8$). In 2004, parasite infracommunities of frogs at RFB, EJS, and ICO were the most diverse, while those at PLR, BSF, and RCH were the least diverse. In 2005, PLR, RSF, and RCH consistently had the lowest infracommunity diversities relative to the other localities.

Component community similarity

Jaccard's indices for component communities ranged from 0.21 to 0.82 in 2004 and 0.22 to 0.93 in 2005 (Appendix 8). The dendrograms based on Jaccard's similarity in 2004 and 2005 (Figs. 11 and 12) corroborated the differences between RCH and PLR with the other wetlands demonstrated in previous analyses. In addition to BSF in 2004, both PLR and RCH clearly separated from the other localities based on parasite species occurrence, and their component communities were the most dissimilar from the others in 2004 and 2005. The low values for Jaccard's similarity were expected for these localities. Only seven or fewer of the eighteen species found in 2004 and the twenty found in 2005 were present at PLR and RCH.

Environment–species associations

Mean infracommunity species richness was negatively correlated with urban area (Spearman's Rho=-0.86, p=0.01) and agricultural area (Rho=-0.69, p=0.04) at the 500 m scale. Mean parasite numbers were negatively correlated with nitrates-nitrites (Rho=-0.77, p=0.01) and forest area (Rho=-0.78, p=0.01) surrounding the localities. Simpson's Dominance Index was negatively correlated with forest area (Rho=-0.69,

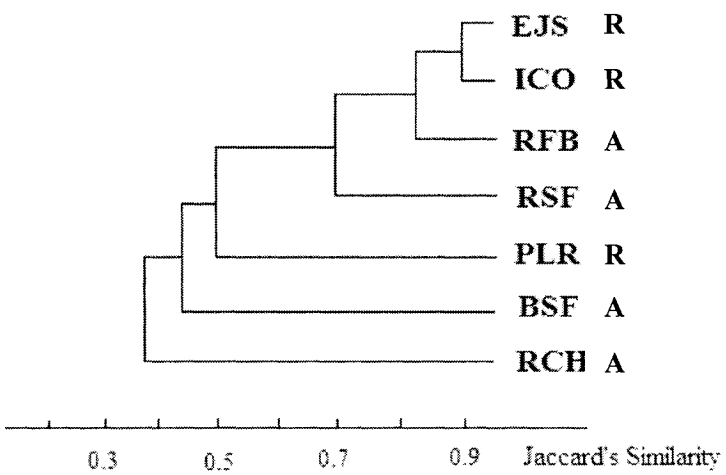

Fig. 11 – Dendrogram resulting from UPGMA cluster analysis on parasite communities of *Rana pipiens* in reference and agricultural localities sampled in 2004. The clusters are based on Jaccard's similarity indices of parasite component communities. R = reference locality, A = agricultural locality. Abbreviations for localities are explained in Table 2.

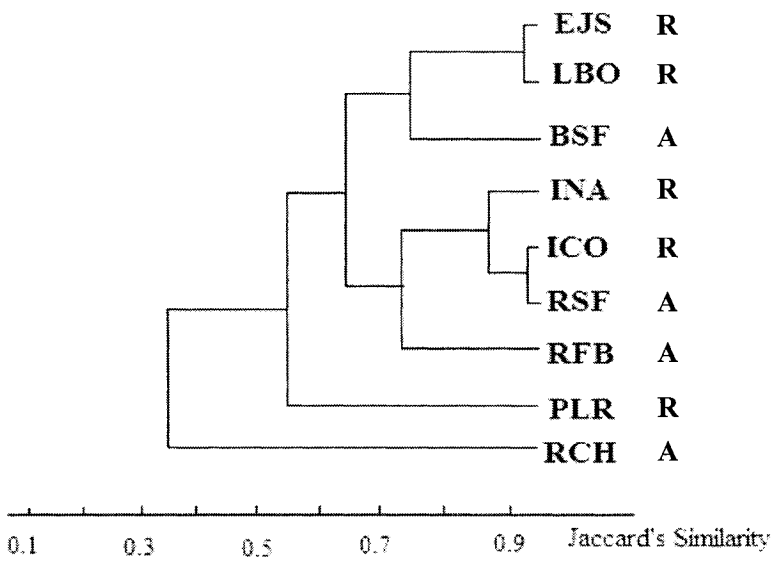

Fig. 12 – Dendrogram resulting from UPGMA cluster analysis on parasite communities of *Rana pipiens* in reference and agricultural localities sampled in 2005. The clusters are based on Jaccard's similarity of parasite component communities. R = reference locality, A = agricultural locality. Abbreviations for localities are explained in Table 2.

p=0.04), and component community species richness was negatively correlated with dissolved organic carbon (Rho=-0.73, p=0.03).

Canonical correspondence analysis

Parasite communities were distinguished further using canonical correspondence analysis. When restricted to taxa with 2% overall occurrence and using the variables dissolved organic carbon and agricultural area (at 500m scale), the CCA model was significant for the first canonical axis (F = 2.880, p = 0.009) and for all four axes (F = 4.254, p = 0.007). Axis 1 explained 57.0% and Axis 2 accounted for a further 43.0% of the variability in parasite species-environment relationships. Overall, the sum of all unconstrained eigenvalues was 1.456 and that of all canonical eigenvalues was 0.917. Axis 1 had the larger eigenvalue (0.523) compared with that of Axis 2 (0.394) and therefore, the former axis defined the clearest gradient. The correlation coefficients showed that Axis 1 reflects a gradient of increasing DOC (r = 0.84). Axis 2 represented a gradient of increasing agricultural area (r = 0.67) (Appendix 9). The model also explained a high percentage of the variability in the abundances for many of the parasite species, but explained <20% of the variability of *Alaria* sp., *A. pipientis, G. quieta, Haematoloechus* spp., *C. dukae, Oswaldocruzia* sp., *Spiroxys* sp. and Spirurida gen. sp. (Appendix 10). The localities were also well represented by the model, with only RFB having a small percent fit (Appendix 11).

Ordination scores for parasite taxa from leopard frogs indicated a positive relationship between most taxa and DOC and a negative correlation with agricultural area (Fig. 13). *Diplostomum* spp., *Fibricola* sp., *Oswaldocruzia* sp., and *R. ranae* had a negative relationship with both DOC and agricultural area. Frogs from localities

surrounded by a high density of agricultural area were characterised by a higher abundance of *Echinostoma* sp., whereas frogs from localities with high DOC were more heavily infected with gorgoderid metacercariae and *Strongyloides* sp.

Parasite communities separated the localities into 3 groups comprising wetlands RCH and PLR (Group 1), wetlands EJS, BSF, and ICO (Group 2), and wetlands INA, LBO, and RFB (Group 3) (Fig. 14). Communities in Group 1 wetlands had a strong positive association with agricultural area; whereas those from Group 2 wetlands had strong positive associations with DOC. Group 3 wetlands had a negative association, more or less, with both environmental variables. The communities in PLR, RCH, RFB, and ICO were positively associated with agricultural area, with the first two wetlands having the strongest relationship with this axis. ICO, BSF, and EJS were positively associated with DOC, and the last two wetlands were also negatively associated with agricultural area. Communities from INA and LBO had the strongest negative relationships with both explanatory environmental variables.

The parasite species composition of wetlands surrounded by agricultural area (Group 1) was dominated by *Echinostoma* sp., whereas the parasite assemblages from Group 2 (EJS, BSF, and ICO) had many co-occurring, mostly trophically transmitted, parasites including *G. quieta, Haematoloechus* spp., Spirurida gen. sp, and *Spiroxys* sp. Gorgoderids and *Strongyloides* sp. were also encountered more frequently at those localities with high DOC. Two direct life-cycle nematodes, *Oswaldocruzia* sp. and *R. ranae*, co-occurred often in Group 3 wetlands along with the larval digeneans *Diplostomum* spp. and *Fibricola* sp.

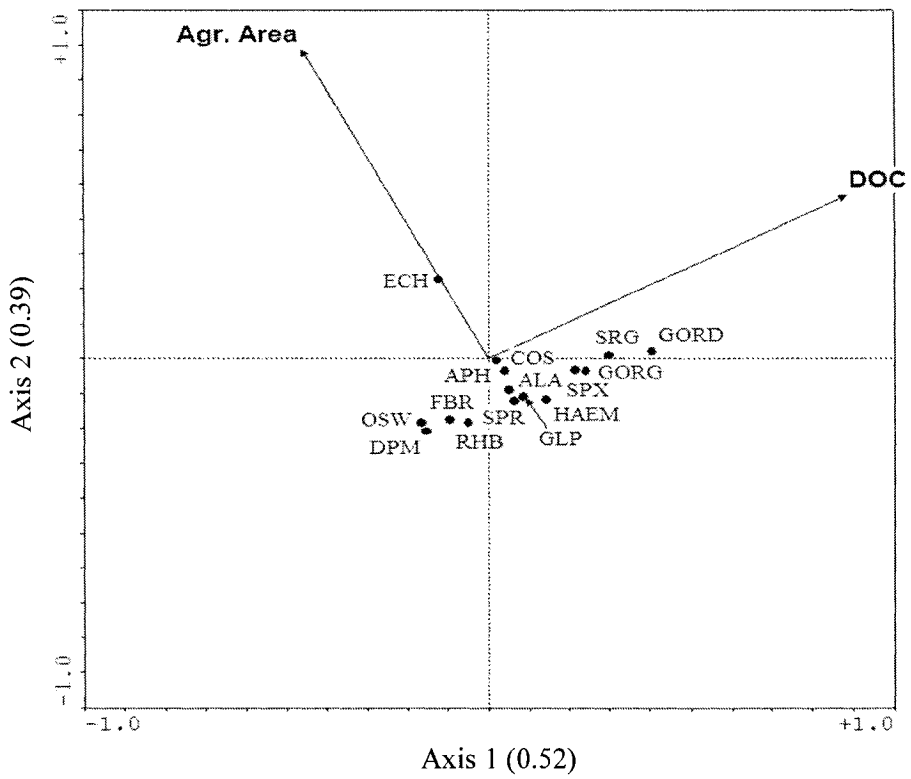

Fig. 13 – CCA species–environment biplots of environmental variables and parasite species abundances in *Rana pipiens* from reference and agricultural localities sampled in July/August 2005. Eigenvalues for Axes 1 and 2 are shown in parentheses. Parasite species abbreviations: ALA = *Alaria* sp.; APH = *Apharyngostrigea pipientis;* COS = *Cosmocercoides dukae;* DPM = *Diplostomum* sp.; ECH = *Echinostoma* sp.; FIB = *Fibricola* sp.; GLP = *Glypthelmins quieta*; GORD = Gorgoderidae gen. sp.; GORG = *Gorgoderina attenuata*; HAEM = *Haematoloechus* spp.; OSW = *Oswaldocruzia* sp.; RHB = *Rhabdias ranae*; SPR = Spirurida gen. sp. SPX = *Spiroxys* sp.; SRG = *Strongyloides* sp. Environmental variable abbreviations: Agr. Area = Agricultural area; DOC = Dissolved organic carbon.

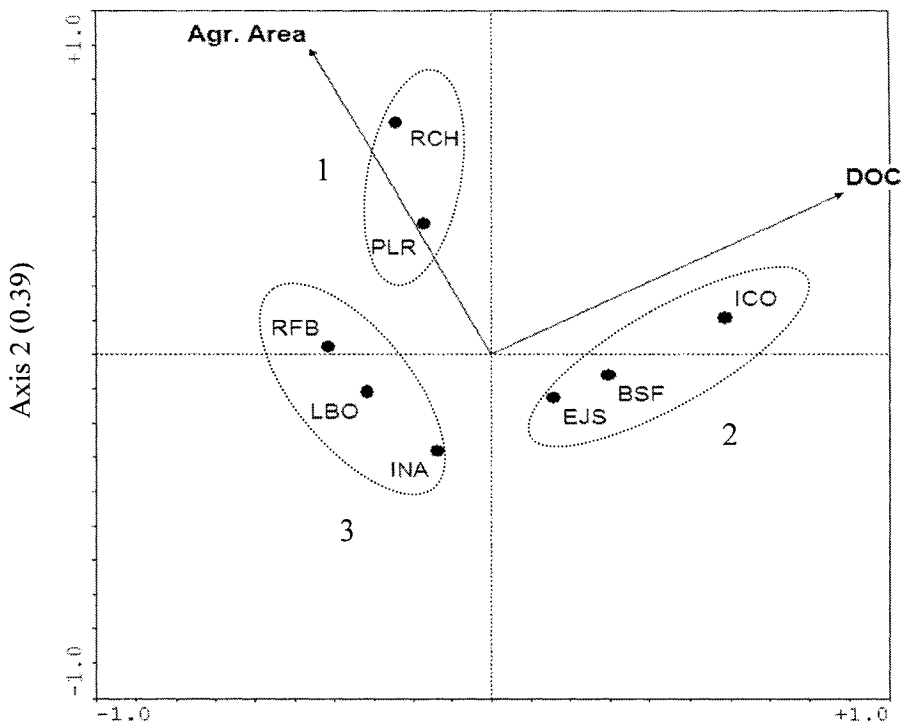

Fig. 14 – CCA locality-environment biplot of environmental variables and parasite species abundances in *Rana pipiens* from reference and agricultural localities sampled in July/August 2005. Eigenvalues for Axes 1 and 2 are shown in parentheses. Environmental variable abbreviations: Agr. Area = Agricultural area; DOC = Dissolved organic carbon. Abbreviations for localities are explained in Table 2.

Discussion

The parasite communities of amphibians have not previously been examined in the context of anthropogenically-disrupted environments. However, other studies using helminth parasite communities of fishes and snails as bioindicators have shown that decreases in species richness and diversity often result from aquatic pollution or development in the surrounding landscape (Lafferty, 1997; Marcogliese, 2004, 2005). A "healthy" environment is most often one that is rich in parasite species (Marcogliese, 2005; Hudson *et al.,* 2006) primarily because species richness and diversity in parasite communities reflect those of the free-living organisms on which parasites depend for transmission and survival (Marcogliese and Cone, 1997b; Marcogliese, 2004, 2005; Hechinger and Lafferty, 2005). Thus, a decrease in the diversity of potential hosts should be accompanied by a reduction in parasite diversity (Hudson *et al.,* 1998). Diversity may also be reduced if free-living stages in parasite life cycles are damaged by environmental contaminants (Poulin, 1992; Pietrock and Marcogliese, 2003). Essentially, parasite species richness and diversity will decline if an environmental disturbance reduces the availability of hosts and parasites or their ability to encounter one another (Poulin, 1992).

Some of the parasite communities of leopard frogs examined in this study appear to have responded to environmental disturbance from indirect and direct effects of agricultural activity. The *a priori* expectation was that the responses in the parasite communities would reflect the dichotomy of wetland types, those receiving and not receiving agricultural run-off. However, this expectation was not generally met. In fact, the most striking observation was the consistent response to disturbance of the parasite communities of frogs from PLR, a reference locality situated within a managed park, and

from RCH, an agricultural locality highly contaminated with pesticides. Compared to other reference and moderately contaminated agricultural localities, the parasite component and infracommunities at PLR and RCH had the lowest species richness and diversity in both 2004 and 2005. This observation was supported by cluster analyses using Jaccard's similarity index which revealed that PLR and RCH were the most dissimilar from other component communities in both years. The parasite communities of two moderately contaminated agricultural localities also showed signs of disturbance, but the responses were not consistent and differed between years. Both the correlation analysis and the CCA attributed the low species richness and the parasite species abundances at these localities to landscape variables associated with the wetlands, and to aspects of their water quality.

Significant negative relationships were found between landscape variables representing human development (urban area and agricultural area) at the 500m scale and species richness of parasite infracommunities. The CCA revealed that the extent of agricultural area surrounding the wetlands was a strong predictor of parasite community structure. On the ordinations, the parasite communities of PLR and RCH were strongly and positively associated with this environmental gradient. These two localities, which had among the lowest infracommunity species richness, were surrounded by the greatest areas of anthropogenically-modified landscape compared to the other wetlands in this study.

Most adult trematodes of amphibians use aquatic or semi-aquatic arthropods as second intermediate hosts (Prudhoe and Bray, 1982). Development and deforestation around freshwater lakes and streams can have significant negative effects on these

invertebrate intermediate hosts. Indeed, Moore and Palmer (2005) found low invertebrate diversity in streams that were surrounded by developed land and had no riparian forest cover. Additionally, in heavily logged boreal watersheds, the low parasite infracommunity richness of northern redbelly dace (*Phoxinus eos*) was attributed to a reduction in the abundance of invertebrate intermediate hosts, particularly mayflies, as a result of the deforestation (Marcogliese *et al.,* 2001b). Loss of physical cover around these wetlands may have affected invertebrate diversity and contributed to the low relative proportion of trophically-transmitted parasites at PLR and RCH. In contrast, the wetlands at RFB and EJS were surrounded by the largest areas of forested land on both the 100m and 500m scales, and frogs from these localities consistently had the greatest relative proportions of trophically-transmitted parasites.

Agricultural or urban expansion can also dramatically change environment use by definitive hosts. Reduction in the use of the area by birds and small mammals should also affect populations of parasite species transmitted by these hosts (Lafferty and Kuris, 2005). More specifically, habitat reduction and/or fragmentation may limit the access of various vertebrate definitive hosts to specific wetlands. Depending upon the extent of the change, a suite of parasite species could be eliminated from the local species pool of particular wetlands, thereby impacting infection levels in intermediate hosts in the life cycle (Kuris and Lafferty, 1994; Lafferty, 1997; Hechinger and Lafferty, 2005). In contrast to PLR and RCH, other localities on the 100m scale were surrounded by forest, were part of more extensive wetlands, or were bounded on one side by a river. PLR and RCH were effectively surrounded by agricultural and urban area at the 100m scale and lacked suitable cover over much of the area on the 500m scale as well. Fragmented

landscape and lack of cover may have precluded many species of mammals, birds, and amphibians from using PLR and RCH.

This observation is supported by the fact that parasite species that ultimately infect birds (e.g. *A. pipientis*, *Clinostomum* sp., and *Diplostomum* spp.) and small mammals (e.g. *Alaria* sp., *Fibricola* sp., and Spirurida gen. sp) and use frogs as intermediate hosts were rarely encountered or absent at PLR and/or RCH. The effects of reduced avian populations as a result of landscape disturbance on the parasite communities of snails have been studied widely (Keas and Blankespoor, 1997; Bustnes and Galaktionov, 1999; Smith, 2001; Huspeni and Lafferty, 2004; Hechinger and Lafferty, 2005). In each case, reduced larval trematode prevalence and species richness in snails was considered to be a direct consequence of changes in habitat use by bird definitive hosts. While it is known that some waterbirds do indeed frequent agricultural wetlands, any fragmentation of these habitats will cause a significant decline in use by these birds, including herons (*Ardea* spp.) (Czech and Parsons, 2002). Herons are definitive hosts for *A. pipientis, Clinostomum* sp., and possibly *Diplostomum* spp. whose larvae have been found in frogs.

There are comparatively few studies examining the implications of reduced mammalian habitat use on the parasite communities of intermediate hosts. However, Koprivnikar *et al.* (2006a) found that total trematode prevalence in grey tree frog (*Hyla versicolor*) tadpoles was strongly associated with agricultural activity, and the prevalence of *Alaria* sp. infection was positively associated with forest cover. The latter was thought to reflect the greater accessibility of forested wetlands to potential canid hosts (Koprivnikar *et al.,* 2006a). In any event, exclusion of definitive hosts from a particular

wetland would reduce diversity of the compound community because without them there would be no source from which intermediate hosts could recruit infections.

Reduced or impaired access to wetlands resulting from habitat fragmentation and isolation can also hamper wetland habitat use by amphibians (Green, 2005) and hence transmission of species between frogs in impacted wetlands. Decreased forest cover and increased road density negatively affect species richness in the amphibian community by impeding movement between wetlands, increasing the risk of predation and other mortality factors, or by making the habitat unsuitable for breeding (Houlahan and Findlay, 2003). Reduced colonisation by certain frog species of particular wetlands or reduction in frog populations in general would limit exchange of parasite species between them. In the present study, the relative proportion of parasites maintained by frog definitive hosts was the lowest at PLR and RCH. Parasite species, including *G. quieta, G. attenuata, Haematoloechus* spp., *C. dukae*, *Oswaldocruzia* sp., *R. ranae*, and *Strongyloides* sp., were rarely encountered or missing from PLR and RCH. The reduced parasite diversity and magnitude of infection of these species at these impacted localities may be a reflection of reduced frog use of these wetland habitats, and thus fewer opportunities for exchange of parasites among individual frogs and ranid species.

DOC was positively associated with component community species richness and was the only water quality parameter found to be a significant predictor of parasite species abundance in the CCA. This suggests that this environmental factor somehow aids in sustaining a highly diversified parasite community. Interestingly, on the species-level ordination, the abundances of parasite species transmitted to frogs via the food chain are positively associated with DOC, indicating that transmission of these parasites

may be facilitated at higher concentrations of DOC. However, the processes underlying the exact relationship are not known. DOC can mediate the density of invertebrates in freshwater systems through trophic pathways (Wetzel, 2001). Allochthonous inputs (e.g. leaves, wood) and the breakdown of detritus contribute to DOC, which is taken up by heterotrophic bacteria, which are in turn consumed by filter-feeding and grazing invertebrates, as well as insect larvae (Meyer, 1994). High DOC can cause shifts in the invertebrate community structure from small invertebrate species to large predatory inverterates and insect larvae (Wissel *et al.,* 2003). This could affect the transmission of parasite species such as *G. attenuata* and *Haematoloechus* spp. that use primarily insect larvae and other aquatic invertebrates as second intermediate hosts. Limited data suggest that snail density is not influenced by DOC levels. Prior to the heavy rainfall event, snail counts taken at localities with low DOC (PLR and RCH) had comparable snail densities to those with high DOC (EJS and ICO). It is thus unlikely that DOC levels had a direct effect on snail communities.

There are few links in the literature between DOC and helminth parasites. However, Goater *et al.* (2005) found that water colour, which is strongly and positively associated with DOC (Wetzel, 2001), was also negatively associated with total phosphorus and chlorophyll-*a* concentrations, and thus primary productivity. Whitefish (*Coregonus clupeaformes*) from lakes with darker water colour and low productivity had low parasite intensities and lacked the acanthocephalan infections found in other lakes (Goater *et al.,* 2005). No correlation was found between phosphorous and DOC in the current study and unlike Goater *et al.* (2005), localities with high DOC levels had high parasite diversity.

Concentrations of agricultural pesticides varied greatly between contaminated localities. At times, the concentrations of atrazine at RCH were ten to one-hundred times greater than those measured at the other agricultural wetlands. Parasite infracommunity species richness and diversity of frogs at moderately contaminated agricultural localities, tended to be comparatively lower than those from reference localities, but not as low as those from PLR and RCH. Thus, it appears that even moderate levels of pesticide contamination may impact parasite communities, although the responses are more difficult to interpret. The responses of parasite communities may be subtle or absent if a disturbance or pollution level is moderate (Marcogliese *et al.,* 2006) and would be difficult to detect in part because the responses of all parasite species are integrated together in community analyses (Lafferty, 1997; Kennedy, 1997; Marcogliese, 2005). On the other hand, parasite communities may only respond noticeably if the disturbance exceeds a certain magnitude.

Temporal variation is frequently encountered when studying parasite community structure (Kennedy, 1993), and can be a problem when the objective is detection of the effects of environmental disturbance (Kennedy, 1997; Marcogliese, 2005). In fact, variation in the structure of parasite communities can be indicative of habitat rehabilitation (Cone *et al.,* 1993; Valtonen *et al.,* 2003; Huspeni and Lafferty, 2004) as well as degradation. For example, in 2001, ICO received pesticide run-off from surrounding corn fields, and atrazine concentrations were as high as 45.7 μg/L and species richness and diversity in the parasite community were comparable to those seen at RCH in that year (King *et al.,* unpublished data). However, in 2005, only trace amounts of atrazine were detected in ICO, and the species richness and diversity at this

locality were comparable to those in the reference localities. These data further suggest that pesticide contamination may indeed have an effect on, or at least contribute to, the responses of parasite communities of frogs and also that long term effects on parasite communities at a particular locality may reflect continual contamination or disturbance of it.

Given that the atrazine concentrations in the pesticide mixtures found in RCH often exceeded 2.0 μg/L, the maximum concentration permissible in freshwater for the protection of aquatic life (CCREM, 1993), it is likely that pesticides were indirectly or directly impacting aquatic invertebrate communities at this locality. However, decreased parasitism in agricultural wetlands, particularly of that observed at RCH may also be due to toxicity of pesticides to free-living stages (Pietrock and Marcogliese, 2003). Koprivnikar *et al.* (2006b) found 200 μg/L of atrazine affected the activity and survival of *Alaria* sp. cercariae and 20 μg/L atrazine reduced the infection success of *Echinostoma* sp. in *Rana clamitans* tadpoles. The results in the present study were consistent with some of those presented in Koprivnikar *et al.* (2006b). *Alaria* sp. was absent in RCH and in BSF in 2004, and also rare in other agricultural wetlands, while infection levels of *Echinostoma* sp. at RCH in 2005 were among the lowest. Further investigations into how specific pesticides and pesticide mixtures affect the free-living stages of other parasites species known to infect amphibians are needed, particularly at concentrations more applicable to realistic field situations.

The relative abundances of direct life-cycle nematodes were consistently lower in frogs from RCH, and *R. ranae* was more abundant in reference wetlands than agricultural wetlands in 2004. These results are contrary to reports from lab studies which

demonstrated that pesticide cocktails containing atrazine can suppress the immunity of leopard frogs sufficiently to increase the establishment of directly transmitted parasites such as *R. ranae* (Christin *et al.,* 2003; Gendron *et al.,* 2003). However, immunosuppression due to pesticide exposure may be a factor influencing trematode parasitism. *Fibricola* sp., which infects frogs by cutaneous penetration, was more abundant in agricultural localities in both years of this study. This parasite species, along with *Echinostoma* sp., one of the most abundant parasite species in RCH, infects frogs at the tadpole stage, when the immune system is most susceptible to suppression from pesticide exposure (Carey and Bryant, 1995; Gilbertson *et al.*, 2003). Trematodes with a similar transmission mode, specifically *R. ondatrae* and *Telorchis* sp., were shown to increase in prevalence in tadpoles exposed in the field to agricultural run-off with pesticides (Kiesecker, 2002). In subsequent lab experiments, both eosinophil counts and infection levels of both trematodes were higher in tadpoles exposed to ≥ 3 μg/L atrazine apparently due to pesticide-induced immunosuppression.

Echinostoma sp. was present at all localities in both 2004 and 2005. It was one of the most common and abundant parasite species in the communities of frogs at RCH and PLR, and along with these two impacted localities, was positively associated with agricultural area in the CCA. Species of *Echinostoma* commonly use frogs as second intermediate hosts (Prudhoe and Bray, 1982; Kostadinova and Gibson, 2000), but this fluke infects a wide spectrum of hosts at different stages in its life cycle. Miracidia infect at least four genera of snails and cercariae infect most snails, fingernail clams, and tadpoles. Adults establish in a variety of birds and mammals (McDonald, 1969; Olsen, 1974). In theory, the presence of highly host-specific parasites will decline when

environmental disturbances cause host populations to be low (Lafferty and Gerber, 2002; Lafferty and Holt, 2003), whereas generalist parasites should persist. This is consistent with the distribution of *Echinostoma* sp. observed in this study where the broad specificity makes it more likely that suitable hosts for *Echinostoma* spp. will exist in impacted environments.

Agriculture and its impacts can affect local wetland ecosystems on many levels. Overall, the results of this study provide a number of insights into the impact of anthropogenic disturbance on the parasites of frogs in wetland habitats. Firstly, the parasite communities of leopard frogs inhabiting wetlands adjacent to intensive agricultural land use were impoverished. A generalist parasite species exhibiting low levels of host specificity throughout all stages of its life cycle was more likely to persist above all others in the parasite communities of frogs from these wetlands. The findings further support the notion that landscape may be influential in structuring parasite communities in aquatic organisms through effects on intermediate host populations in the aquatic ecosystem and on definitive host populations in the surrounding habitat. It is clear that a complement to this study should include a census of mammalian, avian, and amphibian populations to be conducted in the year previous to the study and throughout its duration. This would help link parasite species richness and diversity in amphibians with those in other hosts. Similary work has been done for trematode communities of snails and avian definitive hosts (Hechinger and Lafferty, 2005). Secondly, infection levels were associated with DOC and may have been influenced by pesticide contamination, mainly from the herbicide atrazine. While the former may have facilitated parasite transmission, particularly of those parasites using insect larvae as second

intermediate hosts, the latter environmental contaminant likely limited parasite transmission, particularly at RCH. The effects on snail communities and ultimately on trematode transmission remain open.

Frogs play a pivotal role in the transmission of many parasites within aquatic habitats to terrestrial hosts via the food chain. For this reason, parasites of frogs can reflect environmental conditions and provide insight into the interactions between hosts and the effects of anthropogenic activity on them. Based on the results of this study, the parasite communities of frogs appear to be good sentinel organisms of ecosystem health. However, further examination of the parasite communities of additional frog species, such as *Rana catesbeiana* and *R. clamitans* are necessary. This may reinforce the link between anthropogenic disturbance and the structure of parasite communities of frogs, considering similar investigations had not been conducted prior to the current study. Nevertheless, this study showed that frogs from healthy ecosystems indeed appear to be rich in parasite species, whereas those from severely impacted habitats have reduced parasite species richness and diversity.

CONCLUSION

Overall, twenty parasite species were identified from leopard frogs inhabiting wetlands south and east of Montreal in Quebec, thus providing many species for environmental comparisons. When parasite communities were compared between reference and agricultural wetlands, two wetlands were identified as being significantly impacted by surrounding agriculture. Species richness and diversity of the parasite communities from Parc Le Rocher, a reference wetland situated within a managed park, and Rivière Chibouet, a wetland with heavy pesticide contamination, were consistently low in both 2004 and 2005. Their impoverished parasite communities distinguished them from all other localities. Agricultural activity near the wetland habitats, particularly land use and concentrations of dissolved organic carbon and pesticides were linked with parasite community structure. Landscape variables (agricultural area and urban area at 500m scale) were negatively associated with infracommunity species richness, and the CCA revealed that agricultural area was a strong predictor of parasite community structure. The impoverished parasite communities and low relative proportions of parasites using frogs as definitive hosts in these two aquatic ecosystems may reflect the reduced habitat use by small mammal, birds, and also amphibians due to landscape disturbance. *Echinostoma* sp. was the only parasite found at all localities in both years of this study. According to the CCA, the abundance of *Echinostoma* sp. was positively associated with agricultural area and was dominant in the impacted habitats, PLR and RCH. This suggests that parasites with the fewest host restrictions at all stages of its life cycle will persist over more host-specific parasites in impacted habitats. DOC was associated with component community species richness and was another strong predictor

of parasite species abundance in the CCA. The higher abundances of parasite species transmitted via the food chain at reference and less impacted localities may be explained by the trophic pathway promoted by DOC. DOC and pesticides may have played a role in the low relative abundance of parasites requiring trophic transmission at RCH. Pesticide concentrations at this locality were above national guidelines considered safe for freshwater life, and the elevated levels may have affected cercarial survival and infectivity in some species of trematodes.

The parasites of amphibians proved to be good general indicators of anthropogenically-disturbed environmental conditions. Results herein demonstrate that agriculture and aspects of water quality may be affecting parasite biodiversity and that this likely reflects the effects on the host communities which the parasites depend upon.

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APPENDICES

Appendix 1

Habitat Characteristics of Study Localities

Étang John-Sauro

Location

- A Ducks Unlimited-designated, but privately owned wetland (45° 04.598'N, 73° 09.545'W)
- In Venise-en-Québec

General Description

- A diffuse network of marsh and a mixture of temporary and permanent ponds within a natural forest dedicated to conservation
- Wetland was too large to sample, so only used a subset of area 3909 m^2
- The substrate was thick and muddy and with clumps of dead vegetation and wood debris
- No rocks were encountered
- The water was turbid, still, and brown/orange towards the middle of the wetland
- Water depth increased gradually to just less than 1 metre

Vegetation

- Emergent: cattails (*Typha* sp.), tall grasses (*Phragmites* sp. and *Phalaris* sp.), deciduous trees
- Submergent: Sago pondweed (*Potamogeton* sp.)
- Floating: duckweed (*Lemna* sp.), lily pads, and leaves

Animals

- Deer
- Red-winged black birds, unidentified ducks
- Mudminnows, 5-spine sticklebacks
- Adult green frogs, wood frogs, leopard frog tadpoles, unidentified snake
- Dragonflies, damselflies, monarch butterflies, spiders, amphipods, beetles, leeches, water boatmen, and insect larvae (chironomids and odonates order)
- *Helisoma* sp. snails abundant around cattails

Lac Boivin

Location

- A protected area with no direct agricultural input (45° 24,435'N, 72° 41,723'W)
- Situated in Granby, Quebec

General Description

- Sampling and collecting only conducted in one area of the wetland (5098 m^2) due to the extensiveness, great depth, and inaccessibility of most of the northern wetland
- Substrate was thick, loose, deep, muddy, and replete with dead vegetation, particularly dead cattail pieces
- Gas bubbles and a noxious smell were emitted from the sediment
- Depth increased rapidly, essentially to greater than 1 m approximately 1 m from the wetland's margin, and shallow areas less than 20 cm deep were rare.
- Water was brown and still

Vegetation

- Emergent: *Typha* sp., ferns (*Onodea sensibilis*), and *Sagittaria* sp. were dominant and interspersed within the wetland. Purple loosestrife *(Lythrum salicaria)* dominated the area surrounding the wetland later in the summer
- Submergent: *Ceratophyllum* sp. was the dominant submergent vegetation
- Floating: mixture of *Lemna* sp., *Wolffia* sp., *Spirodela* sp., and larger lily pads

Animals

- A mammal thought to be a groundhog
- Red-winged blackbirds, yellow finches, unidentified gulls, unidentified ducks, Canada geese
- Mudminnows, smallmouth bass
- Bullfrog adults and tadpoles, green frog adults and tadpoles, toad tadpoles
- Annelids, leeches, millipedes, beetles, amphipods, water boatmen, mayflies, insect larvae (mostly chironomids and odonates)
- Large operculate snails, snail egg masses; *Helisoma* sp. and *Promenetus* sp. planorbids were commonly encountered

<u>Île Nid d'Aigle</u>

Location

- One of a series of islands called Les Îles de Sorel along the St. Lawrence River (46° 09,082'N, 73° 00,916'W)
- No proximate sources of pesticides in vicinity
- Located near Berthierville, Quebec

General Description

- Large and deep due to recent rainfall
- Eastern side of wetland of area 880 m^2 was sampled, where leopard frogs were observed previously
- Substrate consisted of loose mud and detritus mostly comprised of dead leaves
- Water depth increased quickly to almost 1 m depth approximately 2 m from the wetland margin
- Water level much lower (15m from original margin) during second visit
- Water was moving slightly due to its proximity to the St. Lawrence River and was cloudy due to flooding

Vegetation

- Grass and deciduous trees lined the edges of the collection area
- Emergent: *Roripa amphibia* and *Sparganium* sp. were scattered throughout the wetland, *Sagittaria* sp. and *Butomus* sp. was scarce and in scattered clumps, and *Phalaris* sp. occurred along the tree margin
- Emergent vegetation was very dominant and submerged due to recent flooding

Animals

- Terns, red-winged blackbirds, herons
- YOY catfish, whitesuckers, crayfish, fish eggs
- Leopard frog tadpoles, adult green frogs
- Beetles, amphipods, annelids, isopods, amphipods, water boat-men, spiders, insect larvae (chironomids and odonates)
- Emergent vegetation dotted with *Pseudosuccinea* sp. and planorbid snails

Parc Le Rocher

Location

- Composed of 2 artificial ponds (45° 38,720'N, 73° 19,857'W)
- Situated within a municipal park (managed landscape) in St.-Amable, Quebec
- Neither pond in contact with agricultural run-off

General Description

- Efforts were focused on the smaller of the two ponds (1802 m^2)
- Ponds were separated by a large grassy piece of land 10 m wide
- Substrate was comprised of dead grass, sticks, roots, dead vegetation, and detritus in both ponds
- Rocks and mud were absent
- Depth of the ponds increased rapidly to greater than 1 m less than 1 m from the margin
- Water was standing, clear (relative to other collection ponds), and brown

Vegetation

- Emergent: ponds were surrounded on three sides by *Phalaris* sp. and *Typha* sp. and on the fourth edge by *Phragmites* sp. *Scirpus* sp.
- Submergent: thick *Potamogeton* sp. mats
- Floating: *Lemna* sp. and *Wolffia* sp. dominant along pond margins

Animals

- Crows, ravens, red-winged blackbirds
- Sticklebacks, mudminnows
- Adult leopard frogs
- Leeches, dragonflies, butterflies, water striders
- Snail egg masses, many dead snails in the larger pond, and newly hatched young physids and planorbids

Île de la Commune

Location

- Temporary wetland located on Île de la Commune, Quebec (45° 37,020'N, 73° 28,446'W)
- One of the Boucherville Islands in the St. Lawrence River near the northeast side of Montreal

General Description

- An area of 20380 m^2 was the focus of collection
- Wetland is separated from St. Lawrence River, but is filled with rainfall and run-off from surrounding corn fields
- Canals run through the wetland drain the cornfields; canals varied in depth
- Canals dry by late summer
- Substrate was soft and muddy or firm with roots
- Water was clear and still

Vegetation

- Emergent: *Sagittaria* sp., *Roripa amphibia, Sparganium* sp, *Phalaris* sp. covered sides of wetland; *Typha* sp. dominant in late summer
- Submergent: *Elodea* sp.
- Floating: *Lemna* sp.

Animals

- Deer, Canada geese, herons, red-winged blackbirds
- Adult leopard frogs and tadpoles, green frogs
- Amphipods, spiders, moths, beetles, annelids, chironomid larvae, water boatmen, odonate larvae, annelids, and isopods
- Snail egg masses; *Bythinia* sp.; *Pseudosuccinea* sp. on the blade tips of vegetation

Ruisseau Fairbanks

Location

- Temporary wetland (45° 01,052'N, 73° 21,604'W) surrounding a small tributary of the Richelieu River, which was straightened and regularly dredged for the purpose of draining agricultural (corn and soy) lands.
- Situated in the town of Notre-Dame du Mont Carmel, Quebec

General Description

- A pipe at the east end of the creek carries water to the Richelieu River
- Excess water from the agricultural field enters the creek through a pipe on the west end of the creek
- An abrupt slope on one side of the creek (greater than 1 m less than 30 cm away from the margin), the middle of the creek is very deep, and the left side is relatively level as it merges with the grassland
- Water level was high and cloudy
- Grassland was flooded making it difficult to find a collection area less than 40 cm deep
- Submerged roots from large trees, branches, and fallen logs
- Substrate was comprised of dead maple leaves and unidentified vegetation
- Thick forest canopy covered the creek

Vegetation

- Emergent: *Butomus* sp. and *Sagittaria* sp. were sparse, *Sparganium* sp. was dominant in the flooded grassland; *Sagittaria* sp. and *Onodea sensibilis* were dominant later in summer
- Submergent: *Ceratophyllum* sp.
- Floating: Lily pads and *Lemna* sp.

Animals

- A dead mole, dog
- Red-winged blackbirds, grackles, yellow finches
- Unidentified fish, fish egg masses
- Green frogs, adult leopard frogs, bullfrogs
- Annelids, isopods, beetles, amphipods, water boatmen, chironomid larvae and mosquitoes
- *Helisoma* sp., *Bythinia* sp., and *Promenetus* sp. were the dominant snail genera; snail egg masses

Rivière St-François

Location

- Nearby and upstream from St. Francois River (46° 06,751'N, 72° 54,790'W)
- On the riverbank of the St. Francois River close to its mouth
- Becomes a wetland during high water season

General Description

- Many channels amongst the vegetation
- Wetland was extensive and deep, so collection was focused on a subset nearest the river of area 18270 m^2
- Due to recent flooding, water was cloudy and at a high level; water level dropped dramatically in late summer, with little water entering wetland from river
- Tree canopy cover was dense close to the land dividing the river and the wetland
- Firm substrate was comprised of detritus, mud, and thin root

Vegetation

- Emergent: *Phalaris* sp., *Sparganium* sp., and *Roripa amphibian* were dominant and *Butomus* sp. and *Sagittaria* sp. were sparse
- Some emergent vegetation was submerged due to the high water level
- Purple loosestrife and *Sagittaria* sp. were dominant vegetation in late summer; clumps of *Typha* sp. were close to the river

Animals

- Deer
- Mallard ducks, red-winged blackbirds
- YOY catfish, breeding carp, whitesuckers, fish eggs
- Toads
- Dragonflies, grasshoppers, mosquito larvae, lady bugs, spiders, beetles, amphipods, insect larvae
- Aquatic snails were very few; fingernail clams were abundant and were large; vegetation tips were dotted with *Pseudosuccinea* sp.; snail egg masses

Baie St-François

Location

- The wetland is expansive covering a large area (46° 05,377'N, 72° 56,540'W)
- Situated north of Sorel, Quebec
- Between the Yamaska River and St. Francois River, and receives water from the former

General Description

- Collection was restricted to a 6360 m^2 area nearest the entrance of the wetland
- Substrate was muddy and thick
- Wetland was recently flooded from heavy rainfall
- Water depth was relatively constant at approximately 40 cm; level dropped dramatically in late summer
- Water was brown, clear, still

Vegetation

- Emergent vegetation: dominant *Sparganium* sp. and *Roripa amphibia,* and scattered *Equisetum* sp., *Acorus* sp. (sweetflag), and *Sagitaria* sp.; irises were rare
- In late summer, vegetation was mostly *Sparganium* sp. and purple loosestrife; soil was dry
- Floating: *Lemna* sp.

Animals

- Cows
- Terns, snipe, unidentified ducks, great blue herons, red-winged blackbirds
- Carp, mudminnows
- Leopard frog tadpoles
- Dragonflies, annelids, mosquito larvae, lady bugs, ostracods, water boatmen, flies, spiders, mosquito larvae, and other insect larvae
- *Promenetus* sp, *Helisoma* sp., and *Bythinia* sp.

Rivière Chibouet

Location

- Temporary wetland along a meander of the Chibouet River (a tributary of the Yamaska River) (45° 47,448`N, 72° 49,527`W)
- In St.-Hugues, Quebec

General Description

- The wetland is of area 1453 m^2
- Becomes flooded with river water in early spring, and maintained by rainfall and run-off from surrounding corn fields
- Substrate was organic and muddy with clumps of dead vegetation (particularly cattails) and dead snail shells
- Water was clouded, brown, and the level in the pond recently receded with the water edge greater than 1 m from the vegetation margin
- Water depth gradually increased to just less than 1 m in the middle; less than 0.5 m in late July
- Water had shiny surface

Vegetation

- Emergent: *Typha* sp., *Phragmites* sp., *Sparganium* sp. lined the edges of the wetland
- Submergent: *Centrophyllum* sp., thick mats of *Potamogeton* sp.
- Floating: *Lemna* sp.

Animals

- House cats, raccoons
- Red-winged blackbirds
- Sticklebacks, mudminnows, bluntnose minnows, Johnny darters
- Adult green frogs, tadpoles
- Water boatmen, amphipods, chironomid larvae, water beetles, dragonflies, mayflies, grasshoppers, mosquitoes
- Snail egg masses

Appendix 2

Summary of *Rana pipiens* collections at reference and agricultural localities in July/August 2004 and 2005. The number of frogs collected and the dates of collection are indicated for each locality. Abbreviations for localities are explained in Table 2.

Locality	Number of metamorph *R. pipiens*	Collection Date
EJS	32 / 30	29 July 2004 / 26 July 2005
LBO	30	1 August 2005
INA	3, 3, 24	22 July, 27 July, 5 August 2005
PLR	30 / 30	28 July 2004 / 28 July 2005
ICO	30 / 30	2 August 2004 / 22 July 2005
RFB	31 / 30	27 July 2004 / 27 July 2005
BSF	19 / 30	30 July 2004 / 29 July 2005
RSF	30 / 30	6 August 2004 / 4 August 2005
RCH	31 / 30	26 July 2004 / 25 July 2005

Appendix 3

Results of Mann-Whitney U tests revealing temporal differences in parasite species mean abundances at reference and agricultural localities sampled in July/August 2004 and 2005. Abbreviations for localities are explained in Table 2.

Locality	Parasite species	U	p	Direction from 2004 to 2005
EJS	*Alaria* sp.	397.0	0.03	Increase
	Fibricola sp.	261.0	0.001	Increase
	Gorgoderidae gen. sp.	168.5	<0.001	Increase
	Strigeidae gen. sp.	420.0	0.05	Decrease
PLR	*Diplostomum* sp.	337.5	0.02	Decrease
	Echinostoma sp.	170.5	<0.001	Increase
	Rhabdias ranae	375.0	0.02	Increase
	Spiroxys sp.	345.0	0.005	Decrease
ICO	*Clinostomum* sp.	390.0	0.04	Decrease
	Fibricola sp.	86.5	<0.001	Increase
	Gorgoderina attenuata	229.5	<0.001	Increase
	Gorgoderidae gen. sp.	159.0	<0.001	Increase
	Oswaldocruzia sp.	299.5	0.003	Decrease
	Rhabdias ranae	284.0	0.01	Decrease
	Strongyloides sp.	273.5	0.002	Increase
RFB	*Gorgoderina attenuata*	362.5	0.04	Decrease
	Gorgoderidae gen. sp.	271.0	0.004	Decrease
	Haematoloechus spp.	314.0	0.002	Decrease
	Spiroxys sp.	375.5	0.03	Decrease
	Spirurida gen. sp.	45.0	<0.001	Decrease
	Strongyloides sp.	375.0	0.01	Decrease
BSF	*Alaria* sp.	199.5	0.01	Increase
	Apharyngostrigea sp.	131.5	0.001	Increase
	Echinostoma sp.	111.0	<0.001	Increase
	Fibricola sp.	161.5	0.001	Increase
	Gorgoderidae gen. sp.	86.0	<0.001	Increase
RSF	*Echinostoma* sp.	240.5	<0.001	Increase
	Fibricola sp.	32.5	<0.001	Increase
	Gorgoderidae gen. sp.	314.0	0.003	Increase
	Cosmocercoides dukae	333.5	0.02	Decrease
	Strongyloides sp.	259.0	0.002	Increase
RCH	*Echinostoma* sp.	214.5	<0.001	Decrease
	Fibricola sp.	299.0	0.02	Decrease

Appendix 4

Equations

Simpson's Dominance index is calculated by:

$$D = \sum \frac{n_i (n_i - 1)}{N (N - 1)}.$$

where: N = total number of individuals
n_i = total number of individuals found in the ith species

(For the purpose of this thesis, the inverse of D was calculated: $1/D$)

Shannon-Weiner Diversity function is calculated by:

$$H' = - \sum p_i \ln p_i$$

where: p_i = the proportion of individuals found in the ith species

Brillouin's index is calculated by:

$$HB = \frac{\ln N! - \sum \ln n_i!}{N}$$

where: N = total number of individuals
n_i = total number of individuals found in the ith species

Jaccard's index is calculated by:

$$C_j = \frac{j}{a + b - j}.$$

where: j = number of species found in both localities
a = number of species found in locality A
b = number of species found in locality B

Appendix 5

Results of Mann-Whitney U tests comparing parasite species mean abundances between male and female *Rana pipiens* collected in July/August 2004 and 2005.

	2004	2005
Alaria sp.	U=10.5 p=0.29	U=9020.0 p=0.83
Apharyngostrigea pipientis	U=53.0 p=0.87	U=8817.0 p=0.51
Clinostomum sp.	U=1.5 p=0.05	U=9028.5 p=0.61
Diplostomum spp.	U=69.0 p=0.94	U=8836.0 p=0.54
Echinostoma sp.	U=2871.5 p=0.56	U=8629.0 p=0.46
Echinostomatidae gen. sp.	U=5.0 p=0.65	U=8960.5 p=0.28
Fibricola sp.	U=300.5 p=0.88	U=8574.0 p=0.39
Glypthelmins quieta	U=4.0 p=0.53	U=8805.5 p=0.25
Gorgoderina attenuata	U=47.0 p=0.40	U=8976.0 p=0.75
Gorgoderidae gen. sp.	U=691.5 p=0.91	U=8082.0 p=0.09
Haematoloechus spp.	U=111.0 p=0.72	U=8796.5 p=0.31
Cosmocercoides dukae	U=3.0 p=0.20	U=9087.5 p=0.96
Oswaldocruzia sp.	U=100.0 p=0.19	U=8884.0 p=0.57
Rhabdias ranae	U=185.5 p=0.68	U=9034.0 p=0.88
Spiroxys sp.	U=81.0 p=0.44	U=8936.5 p=0.50
Spirurida gen. sp.	U=202.5 p=0.20	U=9094.0 p=0.97
Strongyloides sp.	U=32.5 p=0.12	U=8658.5 p=0.32

Appendix 6

Results of Spearman-rank correlations between snout-vent length and parasite species abundances at each locality in July/August 2004. Significant values are in bold.

	EJS	PLR	ICO	RFB	BSF	RSF	RCH
Alaria sp.	Rho=-0.21 p=0.24	-	Rho=0.04 P=0.85	Rho=-0.30 p=0.10	-	Rho=0.01 p=0.96	-
Apharyngostrigea pipientis	Rho=0.13 p=0.49	Rho=-0.04 p=0.82	Rho=-0.10 P=0.59	Rho=0.10 p=0.60	Rho=-0.07 p=0.78	-	-
Diplostomum spp.	Rho=0.28 p=0.12	Rho=-0.04 p=0.84	Rho=-0.18 P=-0.34	Rho=0.32 p=0.09	-	-	-
Echinostoma sp.	Rho=-0.14 p=0.45	Rho=0.19 p=0.31	Rho=-0.05 P=0.79	Rho=-0.10 p=0.61	Rho=0.12 p=0.61	Rho=-0.17 p=0.36	**Rho=-0.45 P=0.02**
Fibricola sp.	Rho=0.22 p=0.22	-	Rho=-0.09 P=0.65	Rho=-0.20 p=0.29	-	Rho=-0.17 p=0.36	Rho=0.18 p=0.34
Glypthelmins quieta	Rho=0.11 p=0.54	-	Rho=-0.05 P=0.81	-	-	Rho=-0.28 p=0.18	-
Gorgoderina attenuata	Rho=-0.10 p=0.60	-	Rho=-0.12 P=0.52	Rho<0.001 p=0.98	-	Rho=-0.25 p=0.18	-
Gorgoderidae gen. sp.	Rho=0.22 p=0.22	Rho=0.05 p=0.78	Rho=-0.21 P=0.26	**Rho=-0.46 p<0.001**	Rho=0.11 p=0.64	Rho=-0.12 p=0.53	Rho=-0.15 p=0.41
Haematoloechus spp.	Rho=0.23 p=0.21	Rho=-0.31 p=0.09	Rho=0.13 P=0.56	Rho=0.34 p=0.06	Rho=0.14 p=0.58	Rho=0.20 p=0.28	-
Cosmocercoides dukae	Rho=0.08 p=0.65	-	Rho=-0.12 P=0.53	Rho=0.33 p=0.07	-	Rho=-0.09 p=0.64	-
Oswaldocruzia sp.	-	Rho=-0.31 p=0.09	Rho=-0.11 P=0.58	Rho=0.34 p=0.06	Rho=-0.06 p=0.81	Rho=0.16 p=0.41	Rho=0.06 p=0.74
Rhabdias ranae	Rho=-0.27 p=0.14	-	Rho=0.33 P=0.08	Rho=0.10 p=0.60	Rho=0.03 p=0.91	Rho=-0.09 p=0.64	Rho=0.06 p=0.74
Spiroxys sp.	Rho=0.23 p=0.20	Rho=-0.21 p=0.26	Rho=0.11 P=0.56	Rho=0.19 p=0.32	-	Rho=0.08 p=0.69	Rho=0.02 p=0.91
Strongyloides sp.	Rho=-0.18 p=0.31	-	Rho=0.15 P=0.44	Rho=-0.07 p=0.73	Rho=0.36 p=0.12	Rho=0.13 p=0.49	Rho=0.06 p=0.74

Appendix 7

Results of Spearman-rank correlations between snout-vent length and parasite species abundances at each locality in July/August 2005. Significant values are in bold.

	EJS	LBO	INA	PLR	ICO	RFB	BSF	RSF	RCH
Alaria sp.	Rho=-0.97 p=0.61	Rho=0.11 p=0.58	Rho=-0.21 p=0.27	-	Rho=-0.01 p=0.93	**Rho=-0.44 p=0.01**	**Rho=-0.42 p=0.02**	Rho=0.30 p=0.11	Rho=-0.23 p=0.23
Apharyngostrigea pipientis	Rho=-0.10 p=0.61	Rho=-0.27 p=0.15	Rho=0.28 p=0.13	Rho=-0.15 p=0.42	Rho=0.08 p=0.68	Rho=-0.25 p=0.18	Rho=-0.15 p=0.41	Rho=0.04 p=0.84	-
Diplostomum spp.	-	-	**Rho=-0.60 p<0.001**	Rho=-0.04 p=0.84	Rho=-0.24 p=-0.21	Rho=0.32 p=0.09	-	Rho=0.10 p=0.60	-
Echinostoma sp.	**Rho=-0.53 p=0.002**	Rho=-0.10 p=0.60	**Rho=-0.56 p<0.001**	Rho=0.20 p=0.27	Rho=0.06 p=0.76	**Rho=-0.41 P=0.02**	Rho=0.10 p=0.61	Rho=-0.25 p=0.19	Rho=-0.18 P=0.33
Fibricola sp.	Rho=0.08 p=0.67	Rho=0.06 p=0.77	Rho=0.16 p=0.41	Rho=0.10 p=0.59	Rho=-0.11 p=0.58	Rho=-0.20 p=0.29	Rho=-0.16 p=0.39	Rho=-0.23 p=0.23	Rho=-0.12 p=0.54
Glypthelmins quieta	Rho=-0.20 p=0.29	Rho=0.29 p=0.12	-	-	Rho=-0.29 p=0.12	Rho=0.09 p=0.63	Rho=-0.14 p=0.46	Rho=0.27 p=0.15	-
Gorgoderina attenuata	Rho=-0.19 p=0.29	Rho=0.01 p=0.96	**Rho=-0.43 p=0.02**	-	Rho=0.04 p=0.94	Rho=0.12 p=0.59	Rho=-0.20 p=0.28	Rho=-0.10 p=0.60	-
Gorgoderidae gen. sp.	Rho=-0.22 p=0.24	Rho=-0.34 p=0.06	Rho=-0.10 p=0.60	Rho=0.33 p=0.07	**Rho=-0.38 p=0.04**	**Rho=-0.61 p<0.001**	**Rho=0.38 p=0.04**	Rho=-0.16 p=0.40	-
Haematoloechus spp.	**Rho=-0.67 p=0.045**	Rho=0.12 p=0.53	Rho=-0.01 p=0.96	Rho=-0.15 p=0.43	Rho=0.13 P=0.56	Rho=0.16 p=0.40	-	Rho=-0.02 p=0.91	-
Cosmocercoides dukae	Rho=-0.05 p=0.82	Rho=0.32 p=0.09	Rho=0.31 p=0.09	Rho=-0.12 p=0.54	Rho=0.30 p=0.10	Rho=0.06 p=0.76	-	**Rho=0.51 p<0.001**	-
Oswaldocruzia sp.	Rho=0.10 p=0.60	**Rho=0.36 p=0.05**	Rho=0.07 p=0.71	Rho=0.25 p=0.19	**Rho=0.40 p=0.03**	Rho=0.22 p=0.25	Rho=0.29 p=0.12	**Rho=0.49 p=0.01**	Rho=0.29 p=0.12
Rhabdias ranae	Rho=-0.11 p=0.58	Rho=0.16 p=0.40	**Rho=0.57 p<0.001**	Rho=-0.04 p=0.83	Rho=0.19 p=0.33	-	Rho=0.18 p=0.33	Rho=0.14 p=0.45	Rho=0.18 p=0.33
Spiroxys sp.	Rho=0.07 p=0.72	Rho=0.10 p=0.61	Rho=0.27 p=0.15	-	Rho=0.03 p=0.87	Rho=-0.27 p=0.15	Rho=0.20 p=0.29	Rho=0.31 p=0.09	-
Strongyloides sp.	Rho=0.19 p=0.32	**Rho=0.46 p=0.01**	Rho=0.07 p=0.73	-	Rho=0.19 p=0.32	-	Rho=0.18 p=0.33	Rho=-0.07 p=0.71	Rho=0.18 p=0.33

Appendix 8

Jaccard's indices for pairwise comparisons of parasite component communities in *Rana pipiens* collected from reference and agricultural localities sampled in 2004 and 2005. Abbreviations for localities are explained in Table 2.

	Jaccard's Index 2005								
	EJS	LBO	INA	PLR	ICO	RFB	BSF	RSF	RCH
EJS		0.93	0.61	0.53	0.56	0.53	0.73	0.71	0.40
LBO	-		0.58	0.50	0.71	0.58	0.69	0.58	0.38
INA	-	-		0.60	0.81	0.67	0.59	0.88	0.38
PLR	0.39	-	-		0.64	0.50	0.40	0.60	0.33
ICO	0.89	-	-	0.39		0.71	0.42	0.93	0.40
RFB	0.80	-	-	0.50	0.22		0.50	0.77	0.22
BSF	0.39	-	-	0.45	0.39	0.50		0.69	0.46
RSF	0.55	-	-	0.38	0.82	0.76	0.38		0.38
RCH	0.44	-	-	0.21	0.40	0.56	0.42	0.53	
	Jaccard's Index 2004								

Appendix 9

Canonical and correlation coefficients of environmental variables and CCA axes 1-2.

Environmental variable	Canonical coefficients		Correlation coefficients	
	1	2	1	2
Dissolved Organic Carbon	0.89	0.46	0.84	0.35
Agricultural Area (500m)	-0.47	0.88	-0.44	0.67

Appendix 10

Fit of species to canonical axes and percent variance of species distribution explained by CCA model.

Parasite species	% variance explained by model
Alaria sp.	8.73
Apharyngostrigea pipientis	0.93
Diplostomum spp.	57.91
Echinostoma sp	63.41
Fibricola sp.	54.77
Glypthelmins quieta	4.87
Gorgoderina attenuata	38.17
Gorgoderidae gen. sp.	90.92
Haematoloechus spp.	5.82
Cosmocercoides dukae	0.18
Oswaldocruzia sp.	18.30
Rhabdias ranae	75.90
Spiroxys sp.	14.61
Spirurida gen. sp.	4.59
Strongyloides sp.	47.51

Appendix 11

Fit of reference and agricultural localities to CCA model. Abbreviations for localities are explained in Table 2.

Localities	% fit of locality to model
EJS	87.33
LBO	61.15
INA	98.69
PLR	97.18
ICO	99.10
RFB	-5.97
BSF	50.95
RCH	41.18