

## Original article

## Associations between *Ixodes scapularis* ticks and small mammal hosts in a newly endemic zone in southeastern Canada: Implications for *Borrelia burgdorferi* transmission

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## ABSTRACT

Immature *Ixodes scapularis* infestation and *Borrelia burgdorferi* infection of wild small mammals were studied from June to October in 2007 and from May to October in 2008 at 71 study sites in a zone where *I. scapularis* populations and environmental Lyme disease risk are emerging in southwestern Quebec. Seasonal host-seeking activity of immature *I. scapularis* was similar to patterns reported previously in Canada and the USA: nymphal activity peaked in spring while larval activity peaked in late summer. Synchronous activity of nymphs with some larvae was observed in late spring, which could favour establishment of *B. burgdorferi* strains that cause short-lived infections in their hosts. White-footed mice (*Peromyscus leucopus*), deer mice (*P. maniculatus*), chipmunks (*Tamias striatus*), and red squirrels (*Tamiasciurus hudsonicus*) carried 92.0% of the larvae and 94.2% of the nymphs collected. Adult male white-footed mice carried significantly larger numbers of both larval and nymphal *I. scapularis* than other species and classes of small mammals (different demographic groups or physiological status: age, sex, sexual activity). We conclude that seasonality and host association were comparable to previous studies in North America, even in the context of a newly endemic pattern of low infection prevalence and low densities of host-seeking and feeding *I. scapularis* in southwestern Quebec. Our studies suggest that *B. burgdorferi* transmission cycles are focused on adult male mice (which carried 35% of all feeding ticks collected in the study), so control methods targeting this class of hosts may be particularly effective. However, our study also suggested that habitats containing a diverse host structure may dilute transmission cycles by partitioning of nymphal and larval ticks on different host species.

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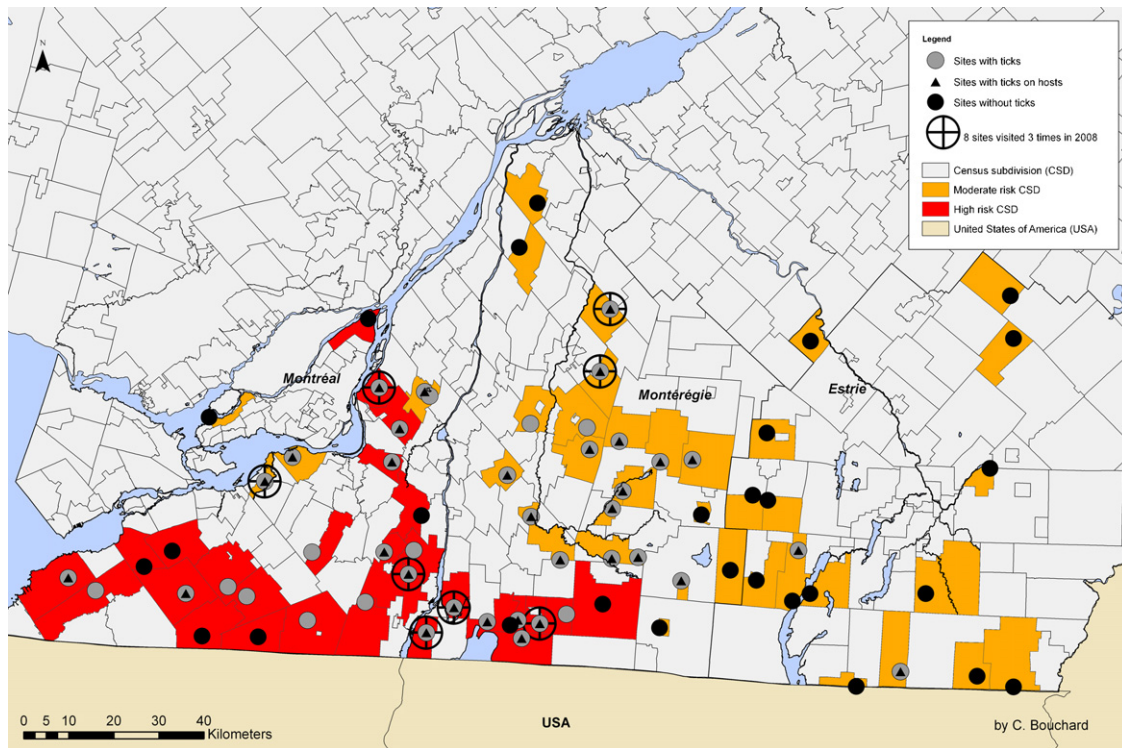
## Introduction

Lyme disease is a zoonosis caused by bacteria of the *Borrelia burgdorferi* sensu lato complex. The tick-borne pathogen *Borrelia burgdorferi* sensu stricto (referred to hereafter as *B. burgdorferi*), the agent of Lyme borreliosis in parts of mid-western and northeastern North America, is transmitted by blacklegged ticks, *Ixodes scapularis* (Acari: Ixodidae) (Spielman et al., 1979; Burgdorfer et al., 1982; Piesman et al., 1986). It is of growing concern in Canada because *I. scapularis* ticks are expanding their geographic range northwards

into southern parts of eastern and central Canada (Ogden et al., 2009, 2010).

Studies suggest that a warming climate may facilitate establishment of blacklegged ticks, carried northwards by migratory birds from endemic areas in the United States of America (USA) and Canada. In Canada, a Lyme disease endemic area has been defined as a locality where the 3 feeding stages of the tick (larva, nymph, and adult) are present for 2 consecutive years, and laboratory results confirm the presence of *B. burgdorferi* in some ticks and/or hosts (Health Canada, 1991). Confirmation of the existence of a new endemic area entails active field surveillance for *I. scapularis* and *B. burgdorferi*. Such surveillance, which involves flagging to collect questing ticks and capture of small mammal hosts to collect blood samples and feeding ticks, was conducted in southwestern Quebec during 2007 and 2008 (Ogden et al., 2008, 2010). These

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**Fig. 1.** Occurrence of *I. scapularis* ticks from the 71 sites visited during the active field surveillance for Lyme disease in southwestern Quebec, 2007–2008.

studies identified the locations of establishing populations of ticks and emerging endemic areas for Lyme disease. They also provided data that are analysed here to explore to what extent the ecology of *I. scapularis* in this region of emergence of Lyme disease risk is comparable with that already documented for endemic areas in North America.

In particular, we investigated to what extent the host associations and the seasonality of immature *I. scapularis* were similar to those in previously studied endemic areas, for 3 reasons. The first reason is practical: we want to ensure that any future field surveillance for ticks coincides with seasonal tick activity periods to reduce the likelihood of false-negative results. Second, seasonality of feeding by immature ticks (specifically the synchrony of attachment of larval and nymphal stages) may determine the nature of *B. burgdorferi* transmission cycles and the strains of *B. burgdorferi* that can be supported (Hoen et al., 2009; Ogden et al., 2010). Third, the association of immature ticks with different host species and different classes of host species (i.e. age, sex, sexual activity) can impact on the force of infection of *B. burgdorferi* transmission cycles. An overarching consideration is the relative contribution of reservoir-competent host species to transmission cycles in terms of their density and the numbers of ticks they carry (Ostfeld, 2011). Transmission may be enhanced if nymphal and larval ticks parasitize the same species and demographic groups of host animals (Randolph et al., 1999; Norman et al., 1999; Bown et al., 2003), or diluted if larvae and nymphs are segregated on different species or demographic groups of the same species (Keasing et al., 2009; Bown et al., 2003).

## Materials and methods

### Study areas

Active field surveillance was carried out each month from June to October in 2007 and May to October in 2008 in southwestern Quebec at 71 sites: Forty-six sites were visited in 2007 and

an additional 25 sites was visited in 2008. The 71 sites were located in 3 different regions of Quebec (Montérégie, Estrie, and Montréal) covering 5325 km<sup>2</sup> (Fig. 1). Thirteen of the 46 sites of 2007 were revisited in 2008 to confirm the presence of different stages of *I. scapularis* for 2 consecutive years. Of these, 8 sites, selected on the basis of a high confidence that *I. scapularis* ticks were established at the sites (Ogden et al., 2008), were visited 3 times during 2008 to get a better picture of seasonal tick activity at individual sites. Therefore, we recorded data during first visits in 2007 and 2008 (one single visit per site from June to October in 2007 or from May to October in 2008) and during revisits in 2008 (1 or 3 visits per site from May to October in 2008).

Locations were selected within deciduous forests. Woodland containing maple trees (*Acer saccharum*) were expected to be favourable for *I. scapularis* (Lindsay et al., 1999a). Sites were selected from locations with suitable habitat based on the following criteria: (i) zone of high or moderate risk for *I. scapularis* occurrence in risk maps (see methodology in Ogden et al., 2008); (ii) maple woodlands that, if mixed, comprised predominantly deciduous trees; (iii) having minimal dimensions of continuous woodland of 0.5 km<sup>2</sup>; and (iv) proximity of vehicular access.

### Wild small mammal trapping

At each site, 150 Sherman<sup>TM</sup> live traps were placed in 3 parallel transects of 50 traps each (150 m × 500 m grid) for one or two consecutive days and nights. Traps were placed for two nights if less than 15 *Peromyscus* spp. mice (white-footed mice and deer mice) were captured on the first night. This sample size was required to give adequate statistical power for other study objectives, particularly determination of the geographic scope of establishment of *I. scapularis* ticks and *B. burgdorferi* in southwestern Quebec, which have been published elsewhere (Ogden et al., 2008, 2010). Because this study design means that in some instances site visits

comprised two rather than one night of trapping, rodent densities per visit are expressed as numbers of rodents captured per unit area, per trap night in the results. Traps were bedded with polyester fibre and baited with moistened sunflower seeds. Traps were set along transects at each site at 16:00, were checked each morning at 7:00, and those containing animals were kept warm or cooled (depending on ambient conditions), prior to examination of the animals. All procedures were undertaken with appropriate ethical approval by the Ministère des Ressources naturelles et de la Faune of the Province of Quebec and the Université de Montréal.

#### Blood and tick collection and detection of *B. burgdorferi* infection

Host-seeking ticks were collected by a standard effort of 3 person-hours of dragging a 1 m<sup>2</sup> white cotton flannel within the trapping grid at each site visit.

Feeding ticks were collected from trapped rodents according to the following protocol. Traps containing animals were placed into a 2 L plastic anaesthetic chamber, and animals were anaesthetized by adding 0.5–1.0 ml 99.9% Isoflurane™ to cotton wool placed within the container. Following anaesthesia, animals were carefully removed from their trap within the container. The entire surface area of each animal was examined and attached ticks were removed with forceps and transferred to labelled vials containing 70% ethanol for transport to the Laboratoire de santé publique du Québec (LSPQ), Sainte-Anne-de-Bellevue, for species identification and thereafter to the National Microbiology Laboratory (NML), Winnipeg, for testing for *B. burgdorferi* infection. For *Peromyscus* spp. mice, the following data were collected: species, weight, sex, age, and reproductive condition (sexual activity) of adults. Visual identification of *P. leucopus* and *P. maniculatus* was confirmed by molecular methods performed at the Laboratoire d'écologie moléculaire et évolution at Université de Montréal using species-specific primers in multiplex-PCR as previously described (Tessier et al., 2004). Also for *Peromyscus* spp., age (juvenile, subadult, and adult) was determined by a weight classification as described for *P. leucopus* (Linzey, 1989) and *P. maniculatus* (Martell, 1983). Whenever possible, blood was collected from each mouse with a needle (23 gauge) and syringe directly from the heart or via the infraorbital sinus using 150 µl Natelson™ blood collecting tubes containing EDTA. In 2008, eastern chipmunks and red squirrels were also bled for serological analyses as more than 10% of *I. scapularis* were found on these rodents in 2007. All *Peromyscus* mice, chipmunks and red squirrels were humanely killed after blood and tick collection while all other small mammal species were released after examination for ticks.

Plasma were separated from the blood samples by centrifugation at 2000 × *g* for 6 min and kept frozen (–20 °C) until shipment on dry ice to the NML for testing. All plasma were tested for IgG antibodies to *B. burgdorferi* using an in-house immunofluorescent assay, followed by a Western blot on reactive samples in 2007, and a two-tier ELISA and Western blot assay in 2008, as previously described (Hofmeister et al., 1999). DNA was extracted from larval and nymphal blacklegged ticks removed from hosts and tested for the presence of *B. burgdorferi* using a polymerase chain reaction (PCR) test. DNA was obtained using Qiagen® DNeasy® 96 Tissue kits (QIAGEN Inc., Mississauga, ON, Canada) optimized for recovery of low-copy number DNA from ticks, and extraction efficiency was assessed using primers specific for the tick 5.8S rRNA–28S rRNA intergenic spacer (IGS). DNA was screened for evidence of *B. burgdorferi* infection using a multiplex real-time PCR targeting the 23S rRNA of *B. burgdorferi* as previously described (Courtney et al., 2004). *B. burgdorferi* infection was then confirmed in positive samples using primers targeting the *ospA* gene (Ogden et al., 2006).

#### Statistical analyses

Analyses of host infestation parameters were conducted on data from *I. scapularis*-positive locations only (at least one *I. scapularis* tick was found after dragging and/or examination of trapped mammals). Data from all visits (first visits in 2007 and 2008 and revisits in 2008) at *I. scapularis*-positive sites were used in our statistical models.

Statistical analyses investigated whether or not we could reject 3 null hypotheses: (i) that there were no differences amongst host species in the numbers of ticks they carried, (ii) that there were no differences between the different species or groupings of *Peromyscus* (i.e. *P. leucopus*, *P. maniculatus*, or *Peromyscus* species) in the numbers of ticks carried, and (iii) within each *Peromyscus* species that there were no differences amongst individuals of different demographic groups or physiological status (age, sex, reproductive status) in the numbers of ticks that they carried.

Host infestation by *I. scapularis* was analysed using mixed effects negative binomial regression models in SAS v. 9.2 (Cary, N.C.) (PROC GENMOD). In these models, the outcome variable was the count of either larvae or nymphs on the captured rodents. Models were fitted using generalized estimating equations using site number as a random effect to account for clustering of rodents by site.

We divided our data into different subsets for analyses, because different variables were collected depending on the host species. For *Peromyscus* spp., which were euthanized, individual animal variables such as their sex, age (based on a weight classification), sexual activity (reproductive status) were included as explanatory variables in our models. In contrast, other host species had less or irregular data recorded depending on duration of anaesthesia and therefore, no individual variables were tested for those species. For all 3 hypotheses, separate analyses were performed for larval and nymphal tick infestations because these stages are often active at different times of the year. In all models, we investigated season, and, for convenience, seasonality was defined and grouped as a variable with 3 categories (spring: May and June; summer: July and August; and autumn: September and October). In all models, we also accounted for any differences between years of sampling, but because some sites visited in 2008 were sites that had been selected for revisits in 2008 on the basis of a relatively high density of ticks a three-category variable was used: data from 2007 first visits, data from 2008 first visits, and data from 2008 revisits. Rodents in the latter category would be expected to carry particularly high numbers of ticks, so in the results, we present only differences between 2007 first visits and 2008 first visits, which would reflect interannual variations in tick abundance.

In our analyses of infestation of *Peromyscus* species, we also hypothesized that any effect of sex would be more pronounced in adult rodents, which may be more active than juveniles resulting in a higher chance of contact with ticks and which may be less resistant to ticks due to higher circulating testosterone levels in adult males (as discussed in Bown et al., 2003). Therefore, we investigated interactions between age and sex in these models. Standard regression diagnostic methods (PROC REG in SAS) were used to assess model fit and multicollinearity amongst explanatory variables.

The explanatory variables included in the 5 statistical models were:

Model 1 (all host species): season, year, and species.

Model 2 (*Peromyscus* spp. subset): season, year, species, sex, age (based on weight), and interaction term between sex and age (sex\*age).

Model 3 (*P. leucopus* subset): season, year, sex, age, and sex\*age.

Model 4 (*P. maniculatus* subset): season, year, sex, age, and sex\*age.

**Table 1**  
Wild small mammals captured and examined for ticks in southwestern Quebec, 2007–2008.

Common name	Species	2007	2008	No. captures	No. sites where captured
White-footed mouse	<i>Peromyscus leucopus</i>	498	345	843	61
Deer mouse	<i>Peromyscus maniculatus</i>	147	308	455	47
<i>Peromyscus</i> mouse	<i>Peromyscus</i> spp.	65	0	65	13
Short-tailed shrew	<i>Blarina brevicauda</i>	71	114	185	45
Sorex shrew	<i>Sorex</i> sp.	42	94	136	37
Red-backed vole/rock vole	<i>Myodes gapperi</i> / <i>Microtus</i> sp. <sup>a</sup>	115	55	170	37
Meadow/woodland jumping mouse	<i>Zapus hudsonicus</i> / <i>N. insignis</i>	27	68	95	27
Chipmunk	<i>Tamias striatus</i>	65	103	168	44
Red squirrel	<i>Tamiasciurus hudsonicus</i>	16	23	39	20
Stoat	<i>Mustela erminea</i>	0	2	2	2
Northern flying squirrel	<i>Glaucomys sabrinus</i>	1	0	1	1
	Total	1047	1112	2159	

<sup>a</sup> *Microtus* sp.: n = 2.

Model 5 (adult *Peromyscus* spp. subset): sex and sexual activity (accounting for season and year).

## Results

### Wild small mammal trapping and tick collection

A total of 2159 wild small rodents were captured and examined (Table 1). We captured rodent species of 11 different genera, the most common species being *P. leucopus*, followed by *P. maniculatus* and *Blarina brevicauda*. *P. leucopus* were trapped from 61 sites, *P. maniculatus* from 47 sites, *B. brevicauda* from 45 sites, and *T. striatus* from 44 sites. Seasonal variations in the estimated densities of hosts *Peromyscus* spp. (per night and per km<sup>2</sup>) are shown in Fig. 2.

A total of 890 ticks of 4 species were collected from the small mammals captured. Species collected, in order of abundance, were *I. scapularis* (n = 855), *I. marxi* (n = 20), *I. angustus* (n = 10), and *I. muris* (n = 5). *I. scapularis* was the most numerous species, and collections consisted of 681 larvae, 173 nymphs, and one adult collected from rodents. *I. scapularis* were found on 43 different sites by flagging and/or examination of trapped mammals. Immature *I. scapularis* were found on 293 hosts at 32 sites (224 hosts carried larvae, 103 hosts carried nymphs, and 34 hosts carried both larvae and nymphs).

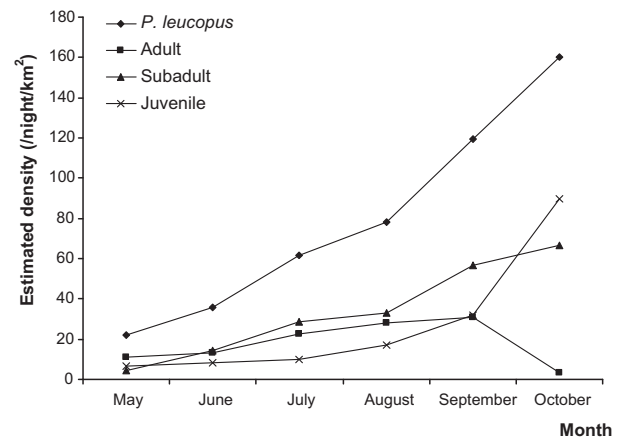
### *B. burgdorferi* infection in wild small mammals and their tick ectoparasites

A total of 7/887 rodents were seropositive for *B. burgdorferi* when considering only the first visit at the 71 different locations. Of these, 2/527 *P. leucopus* and 5/307 *P. maniculatus* were seropositive. For all visits in 2007 and 2008 (including targeted revisits in 2008 where *I. scapularis* or *B. burgdorferi* were more likely to be detected), of the 1169 plasma samples tested, 17 were positive for antibodies to *B. burgdorferi* (see Table 2). From the 9 *P. leucopus* found seropositive, 6 individuals carried ticks (25 larvae and 4 nymphs), but all ticks were PCR-negative for *B. burgdorferi*. From the 6 *P. maniculatus* found seropositive, 3 of them carried ticks (14 larvae and 5 nymphs), and 11 larvae and one nymph were positive by PCR. Six ticks (3 larvae and 3 nymphs) were collected from the one *T. striatus* found seropositive, and one nymph was positive. Finally, on the one *T. hudsonicus* found seropositive, there were 73 ticks collected (65 larvae and 8 nymphs), but none were positive.

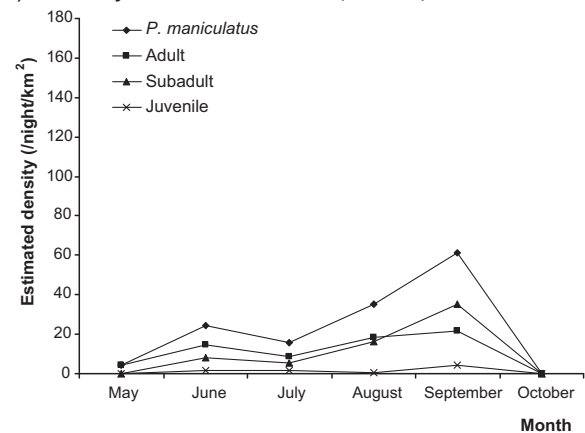
Of the 855 *I. scapularis* collected on rodents, 848 (675 larvae, 172 nymphs, and one adult) were tested by PCR for the presence of *B. burgdorferi* and of these, 39 ticks were positive (22 larvae and

17 nymphs). The positive nymphs were collected from 11 rodents at 6 sites, and the positive larvae were collected from 4 rodents at 3 sites. In total, positive nymphs and larvae were collected from 12 rodents (8 of which were seronegative, 2 of which were seropositive, and 2 of which were undetermined) at 6 different sites.

### i) *Peromyscus leucopus* (n=843)



### ii) *Peromyscus maniculatus* (n=455)



**Fig. 2.** Estimated densities of adult, subadult, and juvenile *Peromyscus* spp. by month (cumulative data from 2007 to 2008 first visits and 2008 revisits). Estimated densities represent the number of captured mice per night per km<sup>2</sup> at each visit. (i) *Peromyscus leucopus* (n = 843); (ii) *Peromyscus maniculatus* (n = 455).

**Table 2**

Number of seropositive rodents for *B. burgdorferi* infection and host infestation by *Ixodes scapularis* collected from wild small mammals captured in southwestern Québec, 2007–2008.

Host species	No. seropositive	No. analysed	No. captured	No. larvae	No. nymphs	No. infested hosts		Mean intensity of infestation (SE)		Range of the intensity of infestation	
						Larvae	Nymphs	Larvae	Nymphs	Larvae	Nymphs
<i>Peromyscus leucopus</i>	9	721	843	403	64	139	48	2.9 (3.1)	1.3 (0.7)	1–18	1–4
<i>Peromyscus maniculatus</i>	6	340	455	129	35	48	20	2.7 (2.4)	1.8 (2.3)	1–11	1–11
Unidentified <i>Peromyscus</i> spp.	0	0	65	0	0	0	0	0.0	0.0	0	0
<i>Blarina brevicauda</i>	0	0	185	18	0	7	0	2.6 (1.7)	0.0	1–6	0
Unidentified shrews ( <i>Sorex</i> sp.)	0	0	136	21	2	6	2	3.5 (5.2)	1.0 (0.0)	1–14	1
<i>Myodes gapperi</i> / <i>Microtus</i> sp. <sup>a</sup>	0	0	170	5	0	5	0	1.0 (0.0)	0.0	1	0
<i>Zapus hudsonicus</i> / <i>N. insignis</i>	0	0	95	4	8	3	8	1.3 (0.6)	1.0 (0.0)	1–2	1
<i>Tamias striatus</i>	1	97	168	20	38	10	21	2.0 (1.9)	1.8 (1.1)	1–7	1–4
<i>Tamiasciurus hudsonicus</i>	1	11	39	75	26	5	4	15.0 (28.0)	6.5 (7.4)	1–65	1–16
<i>Mustela erminea</i>	0	0	2	6	0	1	0	6.0	0.0	6	0
<i>Glaucomys sabrinus</i>	0	0	1	0	0	0	0	0.0	0.0	0	0
Total	17	1169	2159	681	173	224	103	3.0	1.7	1–65	1–16

Host infection by *B. burgdorferi* was described as follows:

Column 2: number of seropositive rodents.

Column 3: number of rodents that were analysed.

Column 4: number of rodents that were captured.

Host infestation by *I. scapularis* was described using the following parasitological indices:

Columns 5–6: number of ticks collected on wild small mammals captured.

Columns 7–8: number of infested hosts with ticks.

Columns 9–10: mean number of ticks per tick-infested host species (mean intensity of infestation with standard error).

Columns 11–12: minimal and maximal number of ticks per host species (range of the intensity of infestation).

<sup>a</sup> *Microtus* sp.:  $n = 2$ .

### Host associations of *I. scapularis*

The distribution of immature *I. scapularis* collected from trapped mammals is summarized in Table 2. Of the 2159 rodents captured, 13.6% of these (293 individual rodents) carried immature *I. scapularis*. These ticks infested rodent species of 10 genera out of 11 different genera captured. Of all animals examined, *P. leucopus* were the most numerous ( $n = 843$ , comprising 39.1% of all captures and from 61 sites).

The 1298 individual *P. leucopus* and *P. maniculatus* represented 60.1% of all the captures. Those 2 species carried 78.1% of all the larvae and 57.2% of all the nymphs collected from captured animals. In comparison, the 207 *T. striatus* and *T. hudsonicus* represented 9.6% of the captures. These 2 species carried 14.0% of all the larvae and 37.0% of all the nymphs collected from captured animals. The highest level of immature *I. scapularis* infestation was 73 ticks (65 larvae and 8 nymphs) on one red squirrel (*T. hudsonicus*).

The seasonal trend in the mean infestation of feeding immature *I. scapularis* collected on rodents is presented in Fig. 3. In most instances, a bimodal pattern of larval host-seeking activity was observed, while a unimodal pattern of nymphal activity was present with peak abundance in June that then tapered off slowly over the summer months over all sites.

### Analysis of the intensity of infestation on *I. scapularis*-positive sites

From all the explanatory variables tested in our models of larval or nymphal counts (number of larvae or nymphs per rodent), season, year of sampling, age, and sex of hosts were statistically significant (details of statistical model results are presented in Table 3).

(i) Model 1: Differences amongst host species in the number of ticks carried.

The numbers of larvae carried by hosts varied significantly with season and host species. Larvae were found more often in autumn (September–October) than in spring (May–June) and also more often in summer (July–August) than in spring. For the

host species effect, we compared all host species against *P. leucopus*, since those mice were a predominant host for immature *I. scapularis*. Accounting for season and year, *P. leucopus* carried significantly larger numbers of larvae compared to all other host species, except *P. maniculatus*. Statistical models investigating nymphal infestations could not be constructed due to the infrequent infestation of many host species with nymphs.

(ii) Model 2: Differences between *Peromyscus* spp. in the number of ticks carried.

Larvae were more abundant on *Peromyscus* spp. in autumn than in spring, and adult mice carried more nymphs than did subadults or juveniles.

(iii) Model 3: Differences amongst *P. leucopus* classes in the number of ticks carried.

There were no differences amongst *P. leucopus* classes in the numbers of larvae carried. Adult *P. leucopus* carried more nymphs than subadults or juveniles, and subadults carried more nymphs than juveniles.

(iv) Model 4: Differences amongst *P. maniculatus* classes in the number of ticks carried.

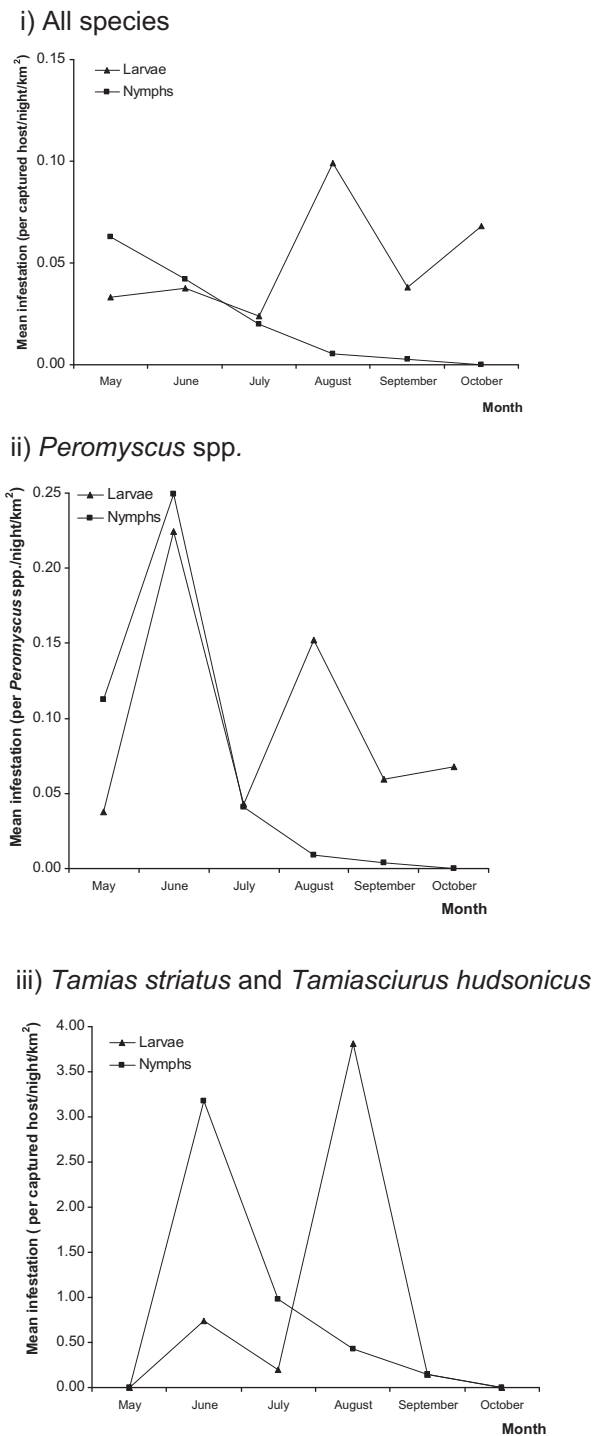
Larval infestations of *P. maniculatus* in 2008 first visits were significantly higher than those during 2007. There were, however, no differences amongst *P. maniculatus* classes in the numbers of larvae carried. Adult *P. maniculatus* carried more nymphs than did subadults, and subadults carried more nymphs than did juveniles.

(v) Model 5: Differences amongst adult *Peromyscus* spp. in the number of ticks carried.

Adult male *Peromyscus* spp. rodents carried more nymphs than females, while adult male *P. leucopus* were infested with more larvae and nymphs than adult female *P. leucopus*.

### Discussion

During these studies, we were able to identify locations of establishing populations of *I. scapularis* ticks and emerging areas for Lyme disease risk in southwestern Quebec. We detected at least one *I. scapularis* tick at 43 sites, and 4 sites fulfilled the criteria for



**Fig. 3.** Seasonality in the mean infestation of captured mammals with immature *I. scapularis* [i.e. average number of ticks per captured host (per night per km<sup>2</sup> at each visit)]. (i) All species; (ii) *Peromyscus* spp.; (iii) *Tamias striatus* and *Tamiasciurus hudsonicus*.

an endemic area according to Health Canada (1991). However, *B. burgdorferi* infection prevalence in hosts or ticks was low at these sites. It should be noted that ticks recently attached to rodents are less likely to be detectably infected than fully engorged ticks while all attached ticks in our study were tested. Therefore, the prevalence of infection in attached ticks detected in our study is likely an underestimate of the true prevalence of infection in ticks that have engorged on small mammal hosts in the region.

The seasonality of *I. scapularis* ticks in southwestern Quebec at the 43 *I. scapularis*-positive sites, and the seasonal patterns of larval and nymphal activities were similar to patterns reported from previous studies in northeastern USA (Kurtenbach et al., 2006): larvae being more abundant on hosts in late summer and nymphs being more abundant on hosts in spring. However, a bimodal pattern of larval host-seeking activity was observed (Fig. 3), while a unimodal pattern of nymphal activity was present with peak abundance in June that then tapered off slowly over the summer months over all sites. This is consistent with studies in northeastern USA (Ginsberg, 1993) and elsewhere in Canada (Lindsay et al., 1999b).

Since larvae do not hatch from eggs until July, the large number of host-seeking larvae and nymphs resuming or commencing activity synchronously in spring may, in part, reflect a cohort of unfed larvae ticks that overwintered. It is assumed that the situation in Quebec with respect to larvae and nymphal activity and overwintering is likely to be similar to that described for Long Point (Lindsay et al., 1999b), and seasonal patterns of cooling in ambient temperatures at higher latitudes curtailing larval activity in autumn (Daniels et al., 1996; Gatewood et al., 2009). Such conditions may be more favourable to transmission of *B. burgdorferi* strains [e.g. RST-3 types (Hanincova et al., 2008)] and other tick-borne bacteria that have short-lived infections in their hosts (Kurtenbach et al., 2006; Ogden et al., 2007). Furthermore, these conditions may promote cofeeding transmission amongst infected nymphs and larvae (Ogden et al., 2007). Infected *I. scapularis* nymphs, rather than persistently infected vertebrate hosts, could be hypothesized as the overwintering reservoir for *B. burgdorferi* in this newly endemic system (Lindsay et al., 1997). Furthermore, environmental conditions influencing the size of overwintering larval and nymphal populations could be critical to the dynamics and maintenance of *B. burgdorferi* cycle (Ogden et al., 2007).

However, based on the bacterial strain found in Quebec area (Ogden et al., 2010), this relative synchronicity between host-seeking larvae and nymphs is probably less important compared to sites in midwest USA (Gatewood et al., 2009). In fact, all but one of the sequence types (STs) that are present in Quebec had previously been found in ticks collected in northeastern USA (Ogden et al., 2010). Further studies are needed to investigate the combined effects of local tick seasonality and stochastic founder events on the diversity of *B. burgdorferi* establishing in this region of Canada.

In some analyses, more larvae were discovered at sites visited in 2008 for the first time compared to sites that were visited in 2007 for the first time. This could reflect increasing abundance of *I. scapularis* in the region as the tick populations become more firmly established, however this difference could also reflect year-to-year variation due to unstudied fluctuations in weather or host densities.

In this study, we focus on small wild mammal hosts that are known hosts of ticks and *B. burgdorferi*. Further studies would be needed to investigate the role of the full host range including white-tailed deer, skunks, raccoons, foxes, coyotes, birds etc. that were not captured by our sampling methods. Of those species captured, immature *I. scapularis* were mostly collected on *P. leucopus*, *P. maniculatus*, *T. striatus*, and *T. hudsonicus*, and those four host species have previously been characterised as being competent reservoirs for *B. burgdorferi* (Mather et al., 1989; Tsao, 2009). Nearly 80% of larvae that we collected were carried by *Peromyscus* spp., and these species carried many nymphs as well (57% of nymphs collected). However, 37% of nymphs were carried by *T. striatus* or *T. hudsonicus* while these species carried only 14% of larvae. Similar results were observed when excluding one outlier *T. hudsonicus* that carried 65 larvae and 8 nymphs, *T. striatus* and *T. hudsonicus* species carried 32% of nymphs. The infestation values for chipmunks, squirrels, or other species than mice are probably quite conservative because their estimate was

**Table 3**

Statistically significant explanatory variables in mixed effects regression models in which the number of immature *I. scapularis* parasitizing captured mammals was the outcome variable.

Outcome	Contrast	Coefficient	SE	p
(i) Differences amongst host species in the numbers of ticks carried				
Larvae	<i>Seasonality</i>			
	Sept–Oct vs. May–June	1.43	0.22	0.02
	July–Aug vs. May–June	1.36	0.20	0.04
	<i>Species</i>			
	<i>P. leucopus</i> vs. <i>B. brevicauda</i>	8.49	5.13	0.0004
	<i>P. leucopus</i> vs. <i>Sorex</i> sp.	4.38	3.09	0.04
	<i>P. leucopus</i> vs. <i>M. gapperi</i> / <i>Microtus</i> sp.	8.88	6.97	0.005
	<i>P. leucopus</i> vs. <i>Z. hudsonicus</i> / <i>N. insignis</i>	15.43	9.05	<0.0001
	<i>P. leucopus</i> vs. <i>T. striatus</i>	3.83	1.37	0.0002
<i>P. leucopus</i> vs. <i>T. hudsonicus</i>	0.21	0.11	0.002	
(ii) Differences between <i>Peromyscus</i> spp. groupings in the numbers of ticks carried				
Larvae	<i>Seasonality</i>			
	Sept–Oct vs. May–June	1.52	0.24	0.008
Nymphs	<i>Age</i>			
	Adults vs. subadults	1.70	0.30	0.005
	Adults vs. juveniles	1.29	0.12	0.006
(iii) Differences amongst <i>P. leucopus</i> classes in the numbers of ticks carried				
Nymphs	<i>Seasonality</i>			
	May–June vs. Sept–Oct	2.02	0.53	0.007
	July–Aug vs. Sept–Oct	1.77	0.41	0.01
	<i>Age</i>			
	Adults vs. subadults	1.26	0.13	<0.0001
	Adults vs. juveniles	2.01	0.35	<0.0001
	Subadults vs. juveniles	1.59	0.35	0.03
(iv) Differences amongst <i>P. maniculatus</i> classes in the numbers of ticks carried				
Larvae	<i>Year</i>			
	2008 (1st visit) vs. 2007 (1st visit)	1.76	0.39	<0.0001
Nymphs	<i>Age</i>			
	Adults vs. subadults	1.68	0.26	0.0007
	Subadults vs. juveniles	0.66	0.12	0.03
(v) Differences amongst adult <i>Peromyscus</i> spp. in the number of ticks carried				
Larvae	<i>Sex (P. leucopus)</i>			
	Males vs. females	2.72	0.64	<0.0001
Nymphs	<i>Sex (Peromyscus spp.)</i>			
	Males vs. females	2.49	0.96	0.02
	<i>Sex (P. leucopus)</i>			
	Males vs. females	5.28	2.91	0.0025

probably confounded by sampling methodology: (i) mice were killed and thoroughly examined in 2007 and 2008, (ii) chipmunks or squirrels were killed and thoroughly examined only in 2008, (iii) chipmunks and squirrels had dense and thick fur limiting the capacity to see attached ticks, and (iv) other host species were less intensely examined due to anaesthesia length. Low tick abundance prevented more detailed investigation of this observation, but we speculate that partitioning of nymphs and larvae amongst diverse host species could result in a ‘dilution effect’ (Ostfeld, 2011), so higher host diversity could be an inhibitory factor on *B. burgdorferi* establishment in this region (Ogden and Tsao, 2009). Increasing host species diversity could have an amplification effect in some circumstances due to increasing numbers of ticks in the system (Ogden and Tsao, 2009). However, if nymphs and larvae are increasingly segregated on different species with increasing diversity, dilution of transmission cycles may be more likely to occur. As an extreme example, even if all species of a theoretical host community were highly competent reservoirs, transmission cycles could not occur at all where some reservoir host species feed only nymphs while all the other reservoir host species are parasitized only by larvae. This would be so even if tick abundance increased with increasing host species diversity.

Clearly, however, *Peromyscus* rodents are important hosts for ticks and, possibly, *B. burgdorferi* on the sites studied. Adult males of this genus were more likely to carry nymphal ticks and in fact carried 35% (300 of 854) of all host-feeding *I. scapularis* collected in the study. The occurrence and significance of this finding for the ecology of *I. scapularis* is similar for other macroparasites and likely rooted in a combination of behaviour and physiology (Wilson et al., 2002). Lower anti-tick resistance of, or grooming by, sexually active males could be an explanatory factor, but other behavioural characteristics such as age, sex-related dispersal rates, and home range sizes may have made them more likely to encounter ticks (see Bown et al., 2003, for a discussion of these factors). Male mice often have larger home ranges and greater activity rates, potentially exposing them to greater numbers of questing ticks than females (Randolph, 1975; Brunner and Ostfeld, 2008). The significance of this finding is that adult male *Peromyscus* spp. and more specifically adult male *P. leucopus* may contribute more to the transmission of tick-borne pathogens in this location being more likely to encounter infective nymphs, and transmission may be focused on a small proportion of the rodent host population as occurs in transmission cycles of tick-borne zoonoses in Europe (Randolph et al., 1999; Perkins et al., 2003). Special attention should, therefore, be made to these animals when employing control methods (e.g. vaccination or tick control:

Dolan et al., 2004; Tsao et al., 2004). To date, some of these methods have involved trapping mice in Sherman™ live traps (Tsao et al., 2004) and, using this method, control could easily be targeted to adult male mice. However, perhaps there are more efficient methods of targeting adult mice for which further research is needed.

In conclusion, our study indicates that the seasonality of *I. scapularis* ticks in southeastern Canada is mostly consistent with studies in northeastern USA with the caveat of possibly greater synchronicity of larval and nymphal ticks in spring. The latter characteristic may have consequences for the species and strains of *I. scapularis*-borne pathogens that establish in the region. Our study also identifies the possibility for 2 competing influences of tick-host associations on transmission of tick-borne pathogens. Adult males of *Peromyscus* mice carry many larval ticks, but also proportionately more nymphal ticks than other mice classes possibly focusing transmission on a smaller cadre of the mice population. However, squirrels and chipmunks may be 'stealing' infective nymphs from *Peromyscus* species and preventing them from contributing to transmission by virtue of the relatively small number of larvae that these species carry relative to *Peromyscus* spp. Quebec may be in the early stages of establishing tick populations prior to the establishment of efficient *B. burgdorferi* transmission cycles, which could become a high public health risk for Lyme disease (Ogden et al., 2010). Considering the public health significance of the findings, this situation may offer a unique opportunity to investigate the ecology of Lyme disease in an emerging zone.

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