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Tick community composition in Midwestern US habitats in relation to sampling method and environmental conditions

Evelyn C. Rynkiewicz · Keith Clay

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Abstract The ranges of many tick species are changing due to climate change and human alteration of the landscape. Understanding tick responses to environmental conditions and how sampling method influences measurement of tick communities will improve our assessment of human disease risk. We compared tick sampling by three collection methods (dragging, CO_2 trapping and rodent surveys) in adjacent forested and grassland habitats in the lower Midwest, USA, and analyzed the relationship between tick abundance and microclimate conditions. The study areas were within the overlapping ranges of three tick species, which may provide conditions for pathogen exchange and spread into new vectors. *Dermacentor variabilis* (American dog tick) was found using all methods, *Amblyomma americanum* (lonestar tick) was found by dragging and CO_2 trapping and *Ixodes scapularis* (blacklegged deer tick) was found only on rodents. Proportion of each species differed significantly among sampling methods. More ticks were found in forests compared to open habitats. Further, more ticks were collected by dragging and from rodents in hotter, drier conditions. Our results demonstrate that multiple sampling methodologies better measure the tick community and that microclimate conditions strongly influence the abundance and activity of individual tick species.

Keywords *Amblyomma americanum* · *Dermacentor variabilis* · *Ixodes scapularis* · Sampling methods · Microclimate

E. C. Rynkiewicz (✉) · K. Clay
Department of Biology, Indiana University, Bloomington, IN, USA
e-mail: ec.rynkiewicz@ed.ac.uk; ecrynkie@indiana.edu

Present Address:
E. C. Rynkiewicz
Ashworth Labs, Department of Biological Sciences, University of Edinburgh, Kings Buildings, Edinburgh EH9 3JT, UK

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Introduction

The geographic ranges of many tick species are changing and expanding due to climate change and human alteration of the landscape (Leger et al. 2013). Seasonal patterns of tick abundance and regional hot-spots of human disease imply a causal link between environmental conditions and the ecology and behavior of ticks (Dantas-Torres et al. 2012). Landscape cover (Schmidt and Ostfeld 2003) and host community composition (Ostfeld and Keesing 2000) can influence tick densities and pathogen prevalence. However, most prior studies have focused on single tick species even though many habitats contain multiple species. Understanding how variation in relative abundance of tick species is affected by environmental heterogeneity and microclimatic conditions is important for assessing human disease risk (Altizer et al. 2013). Here we present results on microclimate predictors of tick abundance and community composition, and assess methodologies for sampling tick communities from natural habitats in southern Indiana, USA. These approaches provide insights into the distribution and density of ticks in multispecies communities and tick-borne disease risk.

Methods

Description of tick and rodent host species

The tick species sampled here were *Amblyomma americanum* (lonestar tick; hereafter, *Amblyomma*), *Dermacentor variabilis* (American dog tick; hereafter, *Dermacentor*), *Ixodes scapularis* (blacklegged deer tick; hereafter, *Ixodes*) and can vector multiple pathogenic bacteria, viruses, and protozoa (Gage et al. 1995; Apperson et al. 2008; Solberg et al. 1996; Eisen 2007a). The timing and overlap between life stages varies between species, with *I. scapularis* life stages being relatively distinct in their seasonal abundances, and *D. variabilis* and *A. americanum* having more overlap (Ostfeld et al. 1995; Kollars et al. 2000; Eisen 2007b).

The rodent species sampled were *Peromyscus leucopus* (white–footed mouse) and *Microtus ochrogaster* (prairie vole). Both species are reservoirs for many tick-borne pathogens (Anderson et al. 1986; Hofmeister et al. 1998) and can vary in their tick burden (Ostfeld et al. 1995; Rynkiewicz et al. 2013).

Study sites

Multiple sites in southern Indiana, USA were surveyed in 2009 and 2011. Eight sites were sampled one time each in 2009 to elucidate broad patterns of tick distributions while two of these sites (2 and 8) were repeatedly sampled five times in 2011 to measure seasonal patterns of tick abundance and environmental conditions (Table 1). In 2009 we collected ticks by dragging, CO_2 trapping, and rodent trapping from forest and open, grassland habitats. In 2011 ticks were collected by dragging and from rodent hosts in conjunction with detailed measures of temperature and relative humidity. Sampling took place between May and July, with one additional sampling in September 2011, for three consecutive days each sampling period. Locations for all sites are reported in Hawlena et al. (2013).

Table 1 Summary of all ticks and hosts sampled from each of the 8 sites sampled once in 2009 and the 2 sites (2 and 8) sampled repeatedly in 2011

Tick species					Host species	
Site/Habitat		*Amblyomma americanum*	*Dermacentor variabilis*	*Ixodes scapularis*	*Peromyscus leucopus*	*Microtus ochrogaster*
1	Forest	11	21	1	0	0
	Open	0	15	0	0	12
2	Forest	151	26	15	9	0
	Open	121	25	66	6	13
2 - 2011	Forest	1,148	363	45	22	0
	Open	380	106	15	13	4
3	Forest	67	65	1	8	0
	Open	21	4	0	1	0
4	Forest	111	132	0	10	0
	Open	37	55	0	4	0
5	Forest	336	26	1	6	0
	Open	87	20	0	2	3
6	Forest	1	30	0	4	0
	Open	1	8	0	1	0
7	Forest	61	26	21	10	0
	Open	17	3	0	2	2
8	Forest	0	14	5	10	0
	Open	0	10	7	3	0
8 - 2011	Forest	1,239	192	24	21	0
	Open	51	7	0	1	2

Ticks were sampled by all methods in each habitat type

Tick collection and rodent host survey

Sampling grids were established at each site within either forested or open habitats. In 2009 we established three grids (either two forest and one open habitat, or one forest and two open habitats depending on site conditions) at each site, while in 2011 we established two grids at each site (one forest and one open grid). In 2009 grids were 40 × 50 m and in 2011 grids were 50 × 60 m.

Tick sampling was done using three sampling methods. Dragging was conducted each morning and afternoon by dragging a 1 m^2 corduroy cloth around each grid 10 m from the grid perimeter (approximately 280 linear m) so as to not disturb tick-host interactions within the grid. Dragging was conducted by the same investigator for equal amounts of time per grid. CO_2 traps were used to attract questing ticks where one trap was placed 10 m outside the corner of each grid, the maximum distance CO_2 traps have been found to attract ticks (Falco and Fish 1991). The CO_2 traps were re-baited with dry ice and checked for ticks each morning and evening. Rodents were trapped and individually censused for ticks by thoroughly searching their skin and fur with forceps. Each site contained 120 Sherman live traps (3 × 3.5 × 9 in., H.B. Sherman Traps, Tallahassee, FL, USA) which were

equally divided among all grids and evenly-spaced within each grid. Traps were baited each evening and were checked early the next morning. Individual rodents were censused for ticks only at their first capture per sampling period to ensure equal sampling effort. All ticks collected by each method were placed in 70 % ethanol and stored at −20 °C until quantification and identification to species and life stage.

Microclimate data collection

Data loggers (HOBO® Pro v2 Internal Temperature/Relative Humidity Data Logger) were placed in the center of each grid on a wooden platform shaded from the sun to measure temperature and relative humidity every 30 min. Saturation Deficit (SD) was calculated as a more informative metric of microclimate conditions (Randolph and Storey 1999), using the calculation:

$$\mathrm{SD} = \left(1 - \frac{\mathrm{RH}}{100}\right) 4.9463 \mathrm{e}^{(0.0621)\mathrm{T}}$$

where RH is relative humidity and T is temperature (°C). SD integrates relative humidity with how much moisture the air can hold, which depends on temperature (Teel and Fleetwood 1982). High SD describes high desiccation risk, where moisture can be more easily pulled from the surfaces of plants, animals and soil, while low SD indicates that the air is near its saturation point for that temperature (Landsberg and Sands 2011; Needham and Teel 1991; Bertrand and Wilson 1996) .

Statistical analysis

All analyses of habitat type and collection method were conducted with data from 2009 due to broader sampling across sites, while analyses of the relationship between microclimate and tick abundance were conducted on 2011 data, which were collected repeatedly from two sites. Models with collection site included as a random factor had lower AIC values and the results of these mixed models are reported.

Generalized Linear Mixed Models (GLMM) with a binomial distribution were used to compare community composition of ticks collected by each sampling method and in each habitat type. Binomial data were the proportion of *Dermacentor* and non-*Dermacentor* ticks in the sample. Because *Amblyomma* was never found on rodent hosts and *Ixodes* was found only on rodent hosts, this provided a general metric to compare sampling methods.

Microclimate measurements and numbers of ticks collected by dragging or from rodents were summarized by day and relationships between tick abundance and mean temperature, relative humidity and SD were calculated (analyses of the maximum and minimum of these measures were the same as for the means, results not shown). GLMM were utilized and AIC values were used to determine the best-fit model. All analyses were done using R Core Team (2013).

Results

Variation among sites, habitats and sampling methods

Dermacentor was collected at all eight sites, *Amblyomma* was found at seven of eight sites, and *Ixodes* was present at four sites. The two sites re-sampled in 2011 (2 and 8) contained

all three species (Table 1). *Peromyscus* were trapped in both open and forested habitats at all sites, while *Microtus* were trapped only in open habitats.

Amblyomma was collected by dragging and CO_2 trapping, *Ixodes* was only collected from rodent hosts and *Dermacentor* was collected by all methods. There were significant differences in tick community composition between sampling methods ($z_{CO_2} = 11.108$, $z_{Drag} = -11.11$, $z_{Hosts} = 6.13$, $p < 0.0001$) and habitat type ($z = 2.43$, $p = 0.015$, Fig. 1a), and a significant interaction between sampling method and habitat (Table 2). More ticks were collected from forest than open habitats overall ($z = -4.99$, $p < 0.0001$) and with each sampling method (dragging: $z = -3.90$, $p < 0.0001$, CO_2 trapping: $z = -7.47$, $p < 0.0001$, rodent hosts: $z = -2.87$, $p = 0.0041$; Fig. 1b). Tick burdens on hosts in open habitats, where mice and voles co-occurred, did not differ by rodent species ($z = -1.87$, $p = 0.062$). The majority of *Dermacentor* collected by dragging and CO_2 trapping were adults. The opposite was true for *Amblyomma* where immature life stages were most common. Larvae and nymphs were the only life stages found on rodent hosts (Fig. 2).

Tick abundance and microclimate

There was seasonal variation in temperature, humidity, and SD, and the abundances of all tick species. Based on dragging *Amblyomma* and *Dermacentor* reached their highest abundance in early June (Fig. 3a). From hosts, *Dermacentor* reached peak abundance in mid-May and *Ixodes* reached peak abundance in July (Fig. 3b). Temperature and SD also peaked in early June and slowly decreased over the season, with relative humidity showing the opposite pattern (Fig. 3c–e).

Analyses of microclimate effects on tick abundance were conducted with 2011 data only. Open habitats had significantly higher temperature ($z = -4.02$, $p < 0.0001$) and SD ($z = -4.28$, $p < 0.0001$) compared to forest habitats, but there was no difference in relative humidity. For analyses of microclimate effects on tick abundances, all tick species and life stages from forested and open habitats were considered together and the best fitting models used log10-transformed tick numbers (Table 2). There was a significant positive, linear relationship between mean temperature and numbers of ticks collected by dragging ($R^2 = 0.36$, $z = 4.06$, $p < 0.0001$) and from rodent hosts ($R^2 = 0.22$, $z = 2.66$, $p = 0.0079$, Fig. 4a, b). There was a significant negative linear relationship between relative humidity and ticks collected by dragging ($R^2 = 0.37$, $z = -5.02$, $p < 0.0001$), but not for ticks collected from rodents (Fig. 4c, d). Ticks collected by dragging also exhibited a significant positive relationship with SD ($R^2 = 0.37$, $z = 4.12$, $p < 0.0001$, Fig. 4e, f) with no relationship between ticks from hosts and SD. Separate analysis of the two tick species collected by dragging showed the same significant patterns as total ticks. From hosts, *Dermacentor* abundance was significantly positively correlated with temperature ($R^2 = 0.032$, $z = 2.25$, $p = 0.024$), but *Ixodes* abundance was independent of microclimate measures.

Discussion

Community composition and abundance of ticks differed significantly by collection method, indicating that estimates of tick abundance and species composition are contingent on sampling methodologies. In total, more ticks were collected from forest habitats compared to grassy habitats but tick abundance was also affected by microclimatic conditions. Our data emphasize the need for comprehensive and repeated sampling of the tick community by multiple methods for improved prediction of disease risk over space and time.

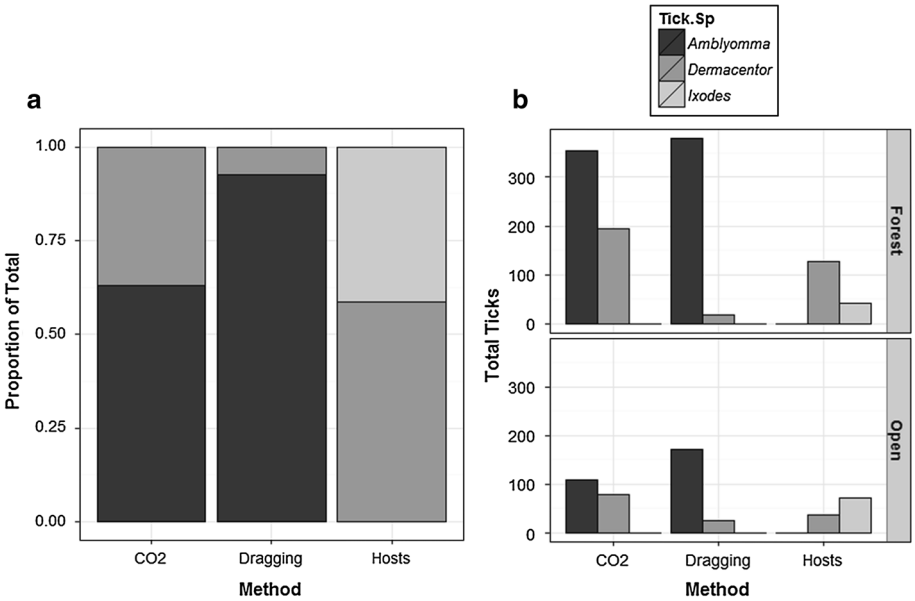

Fig. 1 **a** Proportion of the total ticks collected by each sampling method from each of the three tick species, *Amblyomma americanum, Dermacentor variabilis,* and *Ixodes scapularis*. **b** Tick abundance and community composition from each sampling method, CO_2 trapping, dragging through vegetation, and rodent host survey, in the two habitat types, forest and open grassy field

Table 2 Model comparison for GLMM analysis of community composition (proportion *Dermacentor*) and the relationship between ticks and microclimate measures

Model	dAIC	AIC	df
Proportion Dermacentor, *binomial (Site = Random effect)*			
Habitat + Method + Habitat * Method	0	651.425	7
Habitat + Method	22.5	674.227	5
Microclimate effects on ticks (Site = Random effect)			
log10(SumTicksD) ~ MeanTemp, guassian	0	35.649	3
log(SumTicksD) ~ MeanTemp, guassian	48.4	84.023	3
SumTicksD ~ exp(MeanTemp), gamma with log-link	290.9	326.53	3
SumTicksD ~ MeanTemp, gaussian	302.6	338.212	3
SumTicksD ~ exp(MeanTemp), gaussian	304.7	340.338	3

Temperature and ticks from dragging only are shown. Relative humidity and saturation deficit showed the same outcomes for model comparison. The distribution used in the GLMM was binomial for community composition and is given for each microclimate model

Collection methods

Collection method had a significant effect on which tick species and life stages were sampled, as has been seen with CO_2 trapping (Kensinger and Allan 2011; Falco and Fish 1992). *Amblyomma* was collected by dragging and CO_2 trapping, *Ixodes* was only collected from rodent hosts and *Dermacentor* was collected by all methods. While many ticks are

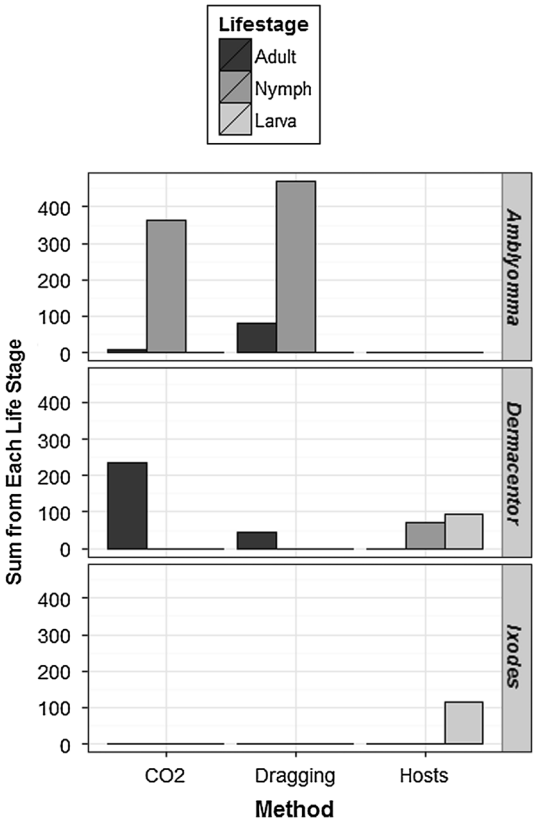

Fig. 2 Abundance of life stages of each tick species (*panels*; *Amblyomma americanum*, *Dermacentor variabilis*, *Ixodes scapularis*) from each sampling method. Larvae were only collected from rodent hosts, while adults were collected only by dragging and CO_2 trapping. Nymphs were collected using all sampling methods

generalists in terms of host use (Klompen et al. 1996), *Amblyomma* was not found here on rodents and may specialize on larger mammals and birds (Kollars et al. 2000; Whitaker 1982). By contrast, *Dermacentor* and *Ixodes* used rodent hosts in both forested and grassy habitats (LoGiudice et al. 2003; Ostfeld et al. 1995). Accurate measurement of tick abundance and community composition is important for assessing local disease risk.

Habitat heterogeneity

Overall, more ticks were collected from forests compared to grassy habitats, similar to what has been previously reported (Ostfeld et al. 1995; Rynkiewicz et al. 2013), and more ticks were collected in forests by every method. However, there was a higher proportion of *Ixodes* on hosts from open habitats compared to forests. Habitat type and vegetation structure can significantly influence host community structure and tick abundance, as where density of bush honeysuckle was correlated with increased density of deer and *A. americanum* (Allan et al. 2010). In our study, grassy habitats were hotter and had higher SD than forests, as in previous studies (Teel and Fleetwood 1982; Bertrand and Wilson

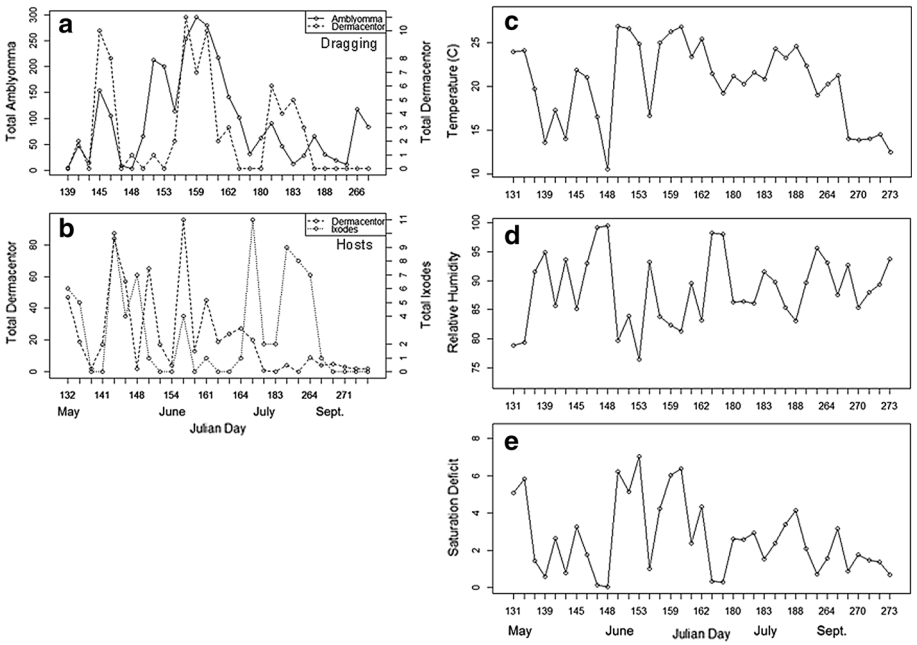

Fig. 3 Seasonal variation in ticks (*Amblyomma americanum*, *Dermacentor variabilis*, and *Ixodes scapularis*) collected from dragging (**a**), rodent hosts (**b**), and microclimate conditions, temperature (**c**), relative humidity (**d**), and Saturation Deficit (**e**) during 2011. Total tick abundance and mean microclimate conditions were summarized per day

1996), but habitats did not differ in relative humidity. Temperature and SD may therefore be more significant environmental variables determining preferred tick habitats (Harlan and Foster 1990; Teel and Fleetwood 1982).

Microclimate conditions

There were stronger relationships between microclimate variables and questing ticks collected by dragging compared to ticks collected from hosts. Questing ticks can respond rapidly to changes in temperature or humidity by moving up and down vegetation or to another location (Needham and Teel 1991), while ticks may remain attached to hosts for days and be carried by them. We therefore expected a weaker association between microclimate and tick abundance on hosts.

There was a positive relationship between tick abundance and temperature, and a negative relationship with relative humidity. Saturation deficit (SD) is an integrated metric of the environment experienced by ticks, and we collected higher numbers of both *Amblyomma* and *Dermacentor* by dragging at high SD, corresponding to hotter, drier conditions. The opposite pattern between SD and abundance of questing ticks has been found in a controlled setting (Randolph and Storey 1999) and in field surveys (Teel and Fleetwood 1982; Perret et al. 2004), where tick abundance was greater under cooler, humid conditions. Differences in tick species' physiological tolerances to dry conditions may account for variance in their response to environmental conditions. The positive

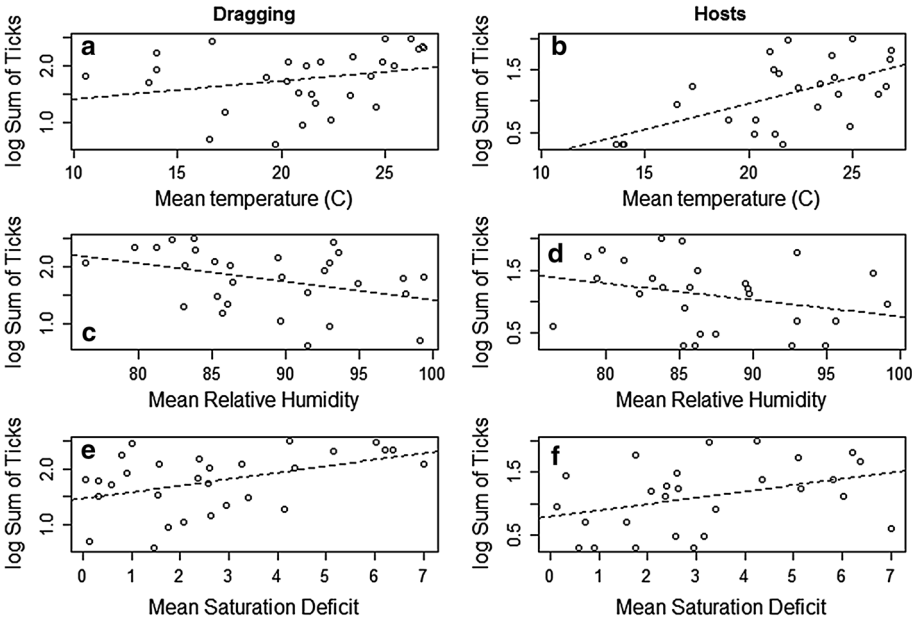

Fig. 4 The relationships between ticks (log10) collected by dragging (*left column*) and on rodent hosts (*right column*) and microclimate variables (temperature, relative humidity and Saturation Deficit). There were significant relationships between dragging ticks and all microclimate measures ($p < 0.0001$ for all). Ticks from hosts only showed a significant relationship with temperature ($p = 0.0079$)

relationship of abundance with SD found here may reflect the conflict between the tick's need to balance water loss by moving to more ideal temperature and humidity conditions and the temperature-dependent nature of tick physiology and activity level (Needham and Teel 1991). Controlled studies of the tick species found at these sites would elucidate how their physiology and behavior respond to temperature and SD.

Conclusions and future directions

Three sampling methods were compared here but there are others such as flagging, pheromone traps, and collections from humans (Barre et al. 1997; Rulison et al. 2013; Schulze et al. 1997). Use of these additional methods could reveal additional details of tick community composition and habitat use, and provide more information on the most efficient sampling techniques for different habitats and conditions. We also sampled only two rodent species but the ticks collected here use a diversity of hosts and differ in host preference (Kollars et al. 1999; Whitaker 1982). Expanding sampling to include more potential hosts may provide insights into which hosts are responsible for the movement of ticks within and among habitats and identification of potential reservoirs for tick-borne pathogens. Lastly, as found here, tick species vary in their phenology and abundance through the year so sampling more sites and at other times beyond the spring and summer peak of tick activity would improve spatial and temporal coverage. Comprehensive sampling will provide data to managers and public health personnel to estimate vector-borne disease risk.

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