

EVALUATING THE USEFULNESS OF THREE INDICES FOR ASSESSING WINTER TICK ABUNDANCE IN NORTHERN NEW HAMPSHIRE

Daniel H. Bergeron¹ and Peter J. Pekins

Department of Natural Resources and the Environment, University of New Hampshire, Durham, New Hampshire 03824, USA.

ABSTRACT: In New Hampshire, winter ticks (*Dermacentor albipictus*) probably have more influence on the moose (*Alces alces*) population than other mortality factors, and predicting the frequency of tick epizootics is an important management consideration. Weather, moose density, and habitat use influence abundance and distribution of winter ticks. We evaluated the usefulness of 3 techniques to index winter tick abundance in 3 regions with variable moose density: 1) flagging for tick larvae, 2) line-transect counts of ticks on harvested moose, and 3) roadside surveys of tick-induced hair-loss on moose. Although counts of tick larvae from fall flagging were not significantly different between years or regions, absolute tick abundance was measurably different (>50%) relative to moose density and years. Tick abundance on harvested moose reflected annual and regional differences; in general, abundance was correlated positively with moose density and annual trends within regions were similar. Tick abundance was highest for calves and lowest for cows. Hair-loss surveys indicated that hair loss was generally related to moose density, and similar annual differences were reflected in all regions. We suggest measuring tick abundance on harvested moose and conducting annual roadside hair-loss surveys to create indices and threshold values useful in predicting an epizootic of winter ticks.

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The winter tick (*Dermacentor albipictus*) is a unique blood-feeding ectoparasite that periodically causes severe mortality in moose (*Alces alces*) populations (Cameron and Fulton 1926–27, Samuel and Barker 1979). It is found in most of moose range in the United States and Canada south of 60° N latitude (Bishopp and Trembley 1945, Wilkinson 1967), but not in Newfoundland or Alaska, although it could presumably survive if translocated (Zarnke et al. 1990, Lankester and Samuel 1998).

Winter ticks have 3 different parasitic life stages: larva, nymph, and adult. Each requires a blood meal to subsequently develop to the next stage, and meals are taken from a single host throughout the

course of one winter (Lankester and Samuel 1998). The life cycle is predictable with little annual variation (Addison and McLaughlin 1988) because its reproductive cycle is dictated by environmental cues such as temperature and photoperiod (Wright 1969, Drew and Samuel 1986). Annual synchrony of the reproductive cycle is partly due to nymphal and adult diapause (Glines and Samuel 1984). Nymphal diapause allows larvae that attach to hosts at different times to be fully developed at the same time (Addison and McLaughlin 1988), and adult diapause allows for synchrony of oviposition (Drew and Samuel 1986). This strict cycle is probably due to the northern climate that allows only a narrow window of reproductive success (Samuel 2004).

¹Present Address: New Hampshire Fish and Game Department, Hazen Drive, Concord, NH 03301, USA.

Weather appears to be the most influential factor of winter tick abundance (DelGuidice et al. 1997, Samuel 2007); however, moose population density seems to influence the distribution and abundance of winter ticks as several studies indicate that tick load increases with moose density (Blyth 1995, Pybus 1999, Samuel 2007). A high density of moose presumably allows for higher larval attachment in autumn, yielding more adult females that produce more eggs (Samuel 2004); evidence for this relationship is mostly correlative. Most larvae climb vegetation in the immediate area of the hatching site and 87% of engorged adult females are found within 60 cm of moose carcasses (Drew and Samuel 1985, 1986), indicating that the drop site of adult female ticks is essentially the site of oviposition. Therefore, distribution of winter ticks is related directly to where adult female ticks drop from moose during early spring (Drew and Samuel 1986, Samuel 2004). Moose in northern New Hampshire preferentially use cut/regeneration habitat in late winter-spring (Scarpitti et al. 2005).

Studies in Canada estimated that average numbers of winter ticks on a single moose were ~30,000 and may exceed 100,000 (Samuel and Barker 1979, Samuel and Welch 1991). High tick loads may lead to several problems including damage and loss of the winter coat, reduced visceral fat stores, restlessness, reduced growth in young moose, and death (Samuel and Barker 1979, McLaughlin and Addison 1986, Samuel 1991, Addison et al. 1994). Studies have found little evidence of anemia in well fed captive moose (Glines and Samuel 1989, Addison et al. 1998); however, the authors speculated that it may occur in wild moose populations on natural diets. Modeling studies conducted by Samuel (2004) and Musante et al. (2010) suggest anemia may have a large impact on moose if it does occur in the wild. Tick induced

hair-loss or alopecia is one of the most common and visual impacts of winter ticks, and rapid hair-loss occurs in March-May, coinciding with engorging by adult ticks (McLaughlin and Addison 1986). Berg (1975) observed high calf mortality in north-western Minnesota when calves died after 2 days of -30°C temperatures and winds of 130 km/h; all dead calves had severe tick infestations and hair-loss. Welch et al. (1990) found that tick-induced hair-loss had little impact on the metabolic rates of captive moose, possibly because of mild ambient temperatures during the study. They speculated that hypothermia is likely unimportant, as severe hair-loss rarely occurs before March, and prolonged severe cold is uncommon thereafter.

Studies by Glines and Samuel (1989) and Addison et al. (1998) found only slight changes in hematologic parameters of well fed captive moose infected with winter ticks. However, the authors speculated that anemia may occur in wild animals on a natural diet. Samuel (2004) and Musante et al. (2007) modeled the impact of different levels of tick infestations and concluded that blood loss associated with moderate to severe infestations of winter ticks would have measurable and substantial impact on energy and protein balance, and cause anemia and possible mortality of moose calves. They predicted that calves with moderate infestations could lose 1–2 times their blood volume during the peak engorgement period; >40% loss of blood volume over a short period of time can cause death (McGuill and Rowan 1989). If these models are correct, winter ticks would likely have less impact on larger adult moose that have larger blood volume and may be in better relative nutritional state in late winter. However, blood loss and/or anemia might negatively affect condition of pregnant cows and post-rut bulls, and although adult moose may be more likely to survive tick infestation,

productivity might decline, particularly in yearling females (Musante et al. 2007, 2010).

Several techniques have been used to estimate the abundance of questing tick larvae and of adult tick loads on moose. Flagging or dragging a sheet over vegetation during the questing period (Piesman et al. 1986, Aalangdong 1994) was used in Elk Island National Park, Alberta to measure the relative abundance of winter ticks in different habitat types to determine whether moose distribution and density in spring dictated distribution and abundance of winter tick larvae (Aalangdong 1994). Digestion of hide samples and subsequent counting of tick exoskeletons provide accurate estimates of tick load (Addison et al. 1979), but may be impractical for managers due to time and cost (Samuel 2007). In Maine, Sine et al. (2009) developed a useful and efficient line-transect method of counting winter ticks on hide samples from harvested moose to estimate/index tick abundance. High sampling rates are possible from harvested moose, but because harvests typically occur during the autumn questing period, onset of winter conditions that would terminate tick activity should be factored to best predict abundance of ticks in spring.

The most common method of indexing winter tick abundance and impact on moose is by conducting hair-loss surveys in late winter (Samuel and Welch 1991, Wilton and Garner 1993). Hair-loss on moose is highly correlated with the rate of grooming against winter ticks (Mooring and Samuel 1999), and annual hair-loss is correlated with the annual tick load (Samuel 2004). Further, years with severe hair-loss coincide with large moose die-offs (Garner and Wilton 1993, Wilton and Garner 1993). Hair-loss surveys conducted since 1984 in Algonquin Provincial Park, Ontario have identified a range of hair-loss severity index

values (HLI) that seem to coincide with moose die-offs (Steinberg 2008).

In New Hampshire, winter ticks probably have more influence on the moose population than disease, predation, habitat, or human-related mortality factors (Musante et al. 2010), and predicting the frequency of tick epizootics is an important management consideration. This study was designed to evaluate the accuracy and potential use of 3 approaches to indexing winter tick abundance and epizootics: 1) flagging for tick larvae, 2) line-transect counts of ticks on harvested moose, and 3) roadside surveys of tick-induced hair-loss on moose.

METHODS

Study Area

Data were collected from 3 northern regions that differed in moose population density (NHFG 2009) (Fig. 1); from highest to lowest density they were CT Lakes (0.83 moose/km², SE = 0.23), North (0.61 moose/km², SE = 0.15), and White Mountains (0.26 moose/km², SE = 0.08), respectively (K. Rines, New Hampshire Fish and Game Department [NHFG], unpublished data). Elevation in the study area ranges from ~120–1900 m, average snow depth ranges from 0–60 cm, and average temperature from –13 to 19 °C (NOAA 1971–2000). The CT Lakes and North regions were dominated by commercial hardwood species including sugar (*Acer saccharum*) and red maple (*A. rubrum*), yellow birch (*Betula alleghaniensis*), and American beech (*Fagus grandifolia*). Red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) tend to be the dominant species at higher elevations (>760 m) and in cold, wet lowland sites (Degraaf et al. 1992). These regions are predominantly forested and the majority of the land is privately owned and commercially harvested using various silvicultural techniques (Degraaf et al. 1992); they contain ~10% wetlands and open water, and

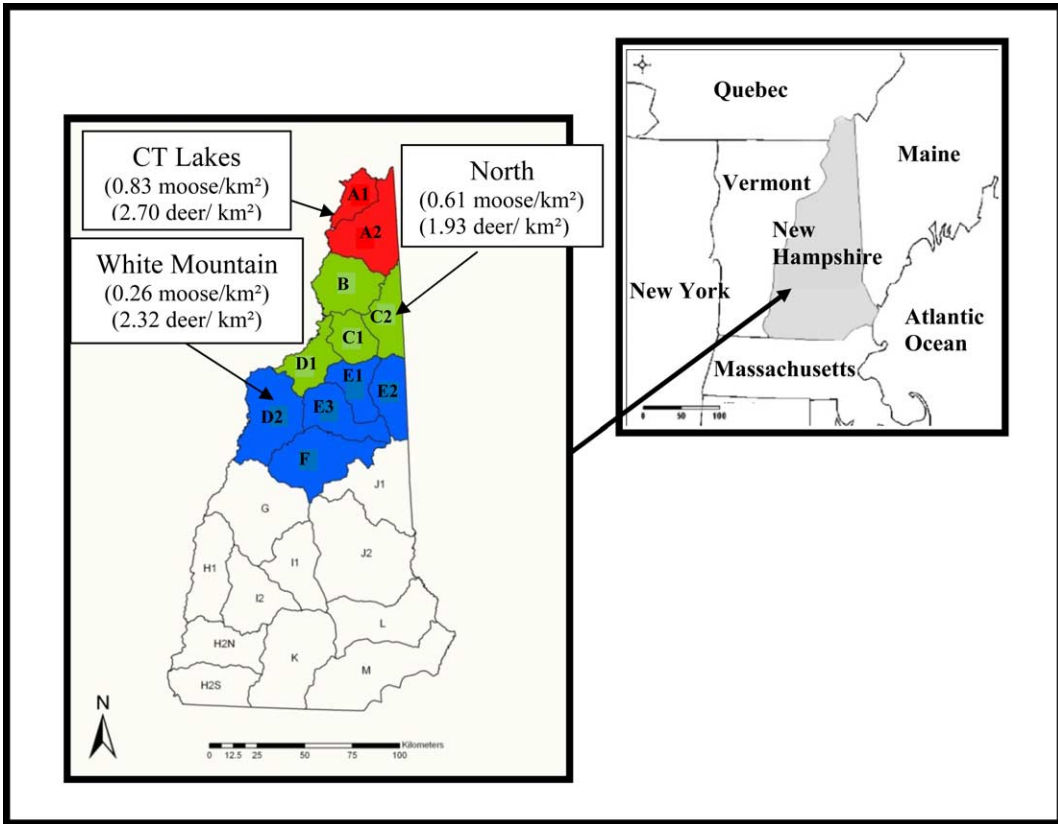


Fig. 1. Three regions in northern New Hampshire with different moose density; the 3 sampling techniques were used in each region to assess the influence of moose density on winter tick abundance, 2008–2010.

are interspersed with trails and logging roads.

The CT Lakes region is hilly with few high mountains, while the North is characterized by high mountainous terrain. The White Mountains region contains the White Mountain National Forest which covers 304,050 ha and is ~97% forested. It contains the highest elevations in New Hampshire and is dominated by beech, sugar maple, and yellow birch; other common species include white ash (*Fraxinus americana*), red maple, red spruce, and eastern hemlock (*Tsuga canadensis*). Timber harvest in this region is done on a smaller scale than the other regions, with maximum clear-cut size of ~10–12 ha (DeGraff et al. 1992, Sperduto and Nichols 2004). White-tailed deer

(*Odocoileus virginianus*) are sympatric with moose throughout the study area, and at moderate to low density (~5/mile²; K. Gustafson, NHFG, unpublished data) (Fig. 1).

Flagging for Tick Larvae

In each region the relative abundance of winter tick larvae was measured during fall in 10–15 clear-cuts ≥4.05 ha (10 acres) and 2–5 years old. Each was sampled every 7–14 days (5–7 times) from 21 September–12 December 2008 and 12 September–3 December 2009. Winter tick larvae were collected by dragging (flagging) a 1 m² white flannel sheet along parallel transects in each cut (Aalongdong 1994). The flannel sheet was attached to a dowel with 2 hose clamps, and held to the side and dragged over the top

of vegetation. New transects were established each visit and separated by 10 m buffers to avoid repeat sampling. Transects were paced to measure length (m) for calculating relative tick density.

The date, time, sample site, and weather were recorded at the beginning of each sampling visit. Each flannel was inspected for tick larvae at the completion of a transect, and if present, was stored in a clear plastic bag, labeled with the date, transect number, and clear-cut ID, frozen within 2 days, and counted at a later date (Aalongdong 1994). Sampling ended in each region when prolonged cold and/or permanent snow pack occurred; such conditions cause winter tick larvae to become inactive or die (Samuel et al. 2000, Samuel 2007).

Ticks were counted by laying the flannel on a white background and recording with a tally counter (Aalongdong 1994). Each tick was removed from the sheet with masking tape to avoid double counts; this process was repeated on the opposite side of the sheet. The relative abundance of ticks per region (ticks/m²) was calculated by tallying the total number of ticks in each region and dividing it by the total transect length sampled. Analysis of variance (ANOVA) was used to detect differences in relative abundance between regions and between sample years. Pairwise comparisons were made with Tukey's test; significance level was set *a priori* at 0.05 for all tests.

Tick Abundance on Harvested Moose

Winter ticks were counted on harvested moose brought to moose check stations operated by the NHFG. Counts were done for the first 5 days of the moose hunt (Saturday-Wednesday) in 2008-2010 at the primary check station in each region: Pittsburg in the CT Lakes, Berlin Fish Hatchery in the North, and Twin Mountain Fish Hatchery in the White Mountain.

Winter ticks were counted *in situ* in 4, 10 × 10 cm sampling plots on a moose carcass: 1) the neck at the base of the skull, 2) the upper edge of the shoulder blade, 3) the rump midway between the hipbone and the base of the tail, and 4) the edge of the rib cage (Bergeron 2011). In each plot ticks were counted on 4 parallel, 10 cm transects roughly 2 cm apart; the fur was combed/held back and all visible ticks were counted along each transect down to the exposed hide (Sine et al. 2009). Only moose that had been harvested within 5 h were sampled because ticks begin leaving a carcass a few hours after death. Time of death, NHFG seal number, and the relative amount of ticks leaving the carcass were recorded at the beginning of each count; biological data and sample region were identified from the seal number.

A 10 × 10 cm hide sample was also cut from each of the 4 plot locations, given hunter permission. Hide samples were initially cut at a larger size then trimmed to 10 × 10 cm, and ticks were then counted on 4 transects on each sample as described above. Each hide sample was labeled with the date, seal number, location of the hide, check station, and then frozen in a sealed plastic bag. Total tick counts were accomplished by digesting the hide samples; each was placed in a 1000 mL beaker with 800 mL of 5% potassium hydroxide solution heated to 90 °C until it was fully digested (~2 h), leaving only the tick exoskeletons intact. The contents were filtered through a 180 µm sieve to separate undigested ticks that were counted under a lighted magnifier (Addison et al. 1979).

Linear regression analysis was used to examine whether the transect counts and hide digestion counts were correlated. This was done to assess the accuracy of performing only transect counts in the field. ANOVA was used to detect differences in transect counts between sample regions, year, and between bulls, cows, and calves. Pairwise comparisons were made with Tukey's test;

significance level was set *a priori* at 0.05 for all tests.

Roadside Surveys of Tick-Induced Hair-Loss on Moose

Weekly hair-loss surveys were conducted from vehicles on predetermined routes in each of the 3 study regions to measure hair-loss on moose, 1 April–1 June 2009 and 19 April–25 May 2010. Routes were chosen to survey traditional roadside salt licks that moose were known to frequent in spring and early summer. Surveys coincided with the periods when nymph and adult winter ticks take blood meals and hair-loss is highest (McLaughlin and Addison 1986, Glines and Samuel 1989); surveys should occur as late as possible because grooming against ticks continues through April (Samuel 2007). The survey dates were adjusted in 2010 because few moose were observed at salt licks prior to 15 April in 2009. Two single-day surveys were also conducted in 2010 to compare with the larger survey. Moose were assigned to 1 of 5 categories of hair-loss: no damage to hair, slight damage (~5–20% hair damaged/lost), moderate (~20–40%), severe (~40–80%), and worst case (>80%). When possible both sides of the moose were observed. However, one-sided examination should provide reliable assessment of tick induced hair-loss as damage is similar on both right and left sides of moose (Samuel and McPherson 2010). Moose were categorized by age and sex, GPS locations, and distinguishing characteristics; digital photographs (not all moose) were also used to help distinguish individual moose to avoid double counting.

Repeat sightings were removed from the analysis by comparing obvious physical characteristics (e.g., antler growth) and photographs when available. Other potential repeat sightings were removed by analyzing GPS locations in ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA).

Buffers of 6.7 km² were placed around each moose location because this area represents the average spring home range of moose in New Hampshire (Scarpitti et al. 2005). If the buffers of 2 locations overlapped and the moose was categorized as the same age, sex, and hair-loss category, it was considered a repeat sighting and removed from the analysis.

An annual hair-loss severity index (HLI) was calculated for each of the 3 sample regions by assigning a number to each hair-loss category (1–5), multiplying the number of moose (M) in each category by that number, then dividing the sum of these numbers by the total (T) number of moose observed (Wilton and Garner 1993, Steinberg 2008):

$$HLI = \frac{(M \times 1) + (M \times 2) + (M \times 3) + (M \times 4) + (M \times 5)}{T} \quad (1)$$

These values were compared to trends in flagging and check station data each year, and HLIs measured in Algonquin Provincial Park, Ontario. A HLI was calculated for bulls, cows, and calves with combined regional data each year to identify differences in HLI by sex/age.

A regional calf:cow ratio was calculated from moose observed in each hair-loss survey. These were compared to ratios calculated the previous fall from moose hunter and deer hunter surveys conducted by NHFG. This exercise was done to investigate whether the proportion of calves declined from fall to spring; measureable calf loss associated with a winter tick epizootic would presumably be identified by a substantially lower calf:cow ratio in spring.

RESULTS

Flagging for Tick Larvae

In total, 17,036 ticks were collected on 11.7 ha of sample transect in 2008, and

11,759 ticks on 17.7 ha in 2009. Ticks ranged, per flagging sheet, from 0–2,212. Although there was no difference ($P > 0.05$) among regions in the number of ticks either year or within regions between years, fewer ticks (~40–75%) were collected in each region in 2009. The average relative density in 2008 and 2009, respectively, was 0.19 and 0.11 ticks/m² (SE = 0.09, 0.04) in the CT Lakes (max = 1.30, 0.63), 0.16 and 0.07 (SE = 0.05, 0.03) in the North (max = 0.62, 0.40), and 0.08 and 0.02 (SE = 0.03, 0.01) in the White Mountains region (max = 0.41, 0.10) (Fig. 2). There was a positive correlation between moose density and tick density in both years ($r^2 = 0.93$ and 0.99).

Although no significant differences were found among regions or between years, absolute differences were large. Mean numbers of ticks declined 42–75% within regions between years, and the mean numbers of ticks were 58 and 82% lower in the White Mountain than CT Lakes regions in 2008

and 2009, respectively (Fig. 2). The mean number of winter ticks collected in individual clear-cuts was below the regional mean in the majority of cuts each year (50–92%) except in the White Mountain in 2008.

Tick Abundance on Harvested Moose

The mean number of ticks (all 4 sampling plots and transects) counted on moose ranged from 25–51 (SE = 6–7), 42–101 (SE = 6–13), and 14–34 (SE = 5–15) in the CT Lakes, North, and White Mountains regions, respectively; highest counts occurred in 2010 in all regions (Fig. 3). All life stages of the tick were observed on moose. The mean number of ticks for combined regional data was 53, 31, and 79 (SE = 7, 4, 9). Tick abundance in the CT Lakes in 2010 was ~2X higher than in 2008 ($P = 0.034$) and 2009 ($P = 0.014$) and in the North was ~1.8X higher in 2008 ($P = 0.034$) and ~2.4X in 2010 ($P = 0.000$) than 2009; tick abundance in the White

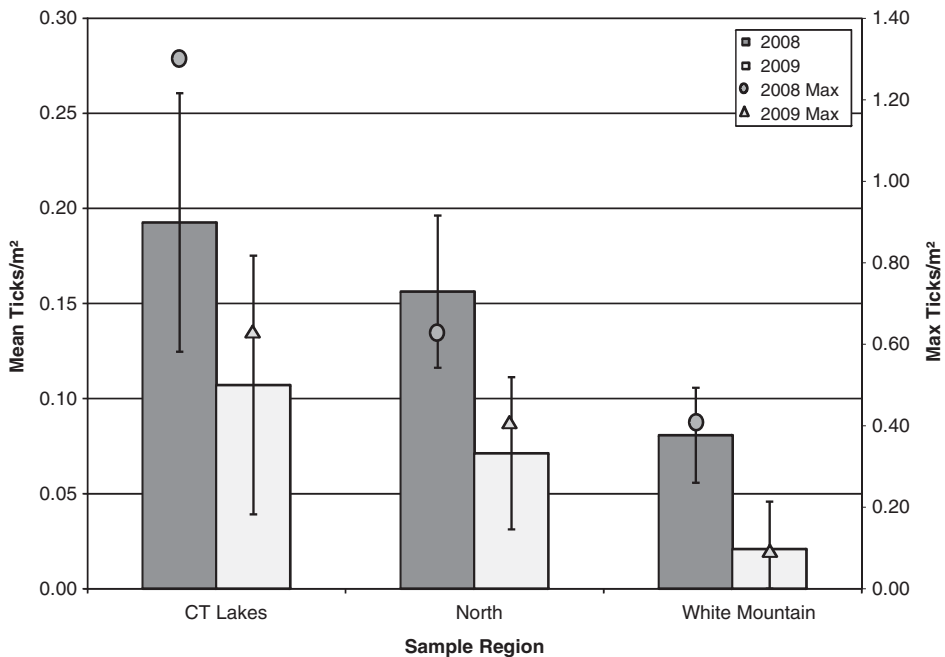


Fig. 2. Mean (\pm SE) and maximum number of winter tick larvae collected while flagging clear-cuts in 3 sample regions of northern New Hampshire, 2008 and 2009.

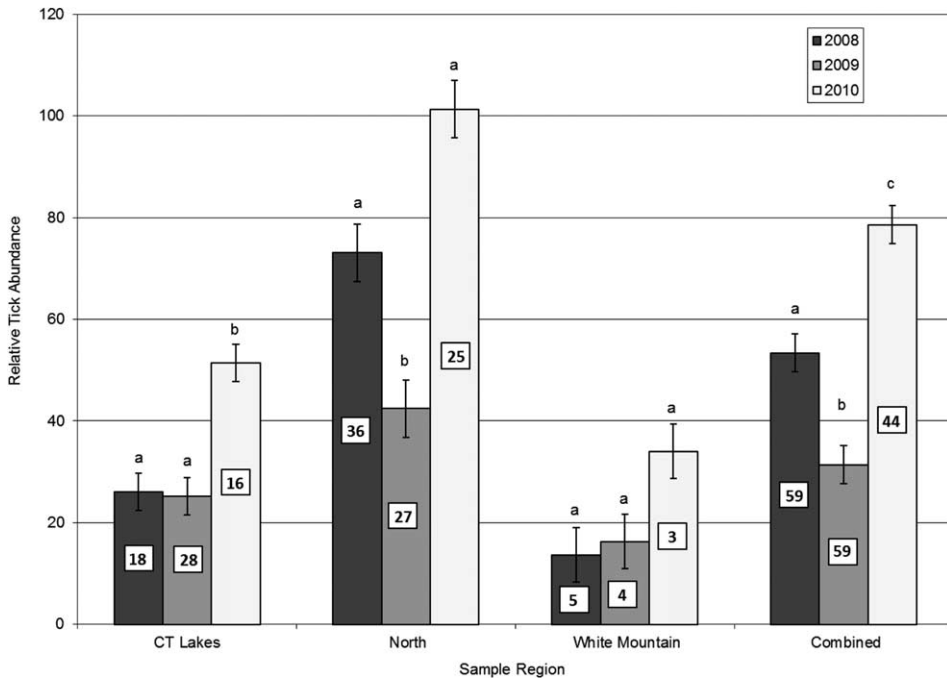


Fig. 3. Mean (\pm SE) number of winter ticks counted on harvested moose in the CT Lakes, North, and White Mountain sample regions, and combined regional data, in northern New Hampshire, 2008–2010. Means are for all 4 areas of the hide and all transects combined. Numbers in columns represent sample sizes. Bars with unlike letters indicate significant differences within sample regions.

Mountain region was not different from other regions or between years. Tick abundance in the North was $\sim 3X$ higher in 2008 ($P = 0.006$) and $\sim 2X$ higher in 2010 ($P = 0.038$) than in the CT Lakes. For all regions combined in 2010, tick abundance was $\sim 1.5X$ higher than in 2008 ($P = 0.032$) and $\sim 2.5X$ higher than in 2009 ($P = 0.000$), and $\sim 1.7X$ higher in 2008 than 2009 ($P = 0.024$) (Fig. 3).

Because regional calf data were minimal, statistical analysis of bull:cow:calf ratios was done using combined regional data. Data were from all 4 sampling plots and transects combined. Calves had more ticks than adult moose each year, and bulls had more than cows (Fig. 4). In 2008, tick abundance on calves was $\sim 2X$ higher than bulls ($P = 0.014$) and $\sim 6X$

higher than cows ($P = 0.000$). Tick abundance on calves was $\sim 4.5X$ higher ($P = 0.004$) than on cows in 2009, and tick abundance on calves and bulls was similar and $>2X$ that on cows in 2010 ($P = 0.013$).

A total of 148 hide samples were collected from 66 moose (26 bulls, 36 cows, 4 calves) in 2008 and 2009; 29, 45, 36, and 38 hide samples were collected from the neck, rib, rump, and shoulder, respectively. The number of ticks per transect was positively correlated with the number of ticks counted for all areas of the digested hide samples; r^2 values ranged from 0.33–0.99. Counts on the rib had the weakest relationship ($r^2 = 0.33$ –0.76), however, sample size was low ($n = 3$ –9); combining regional and yearly rib samples yielded $r^2 = 0.70$.

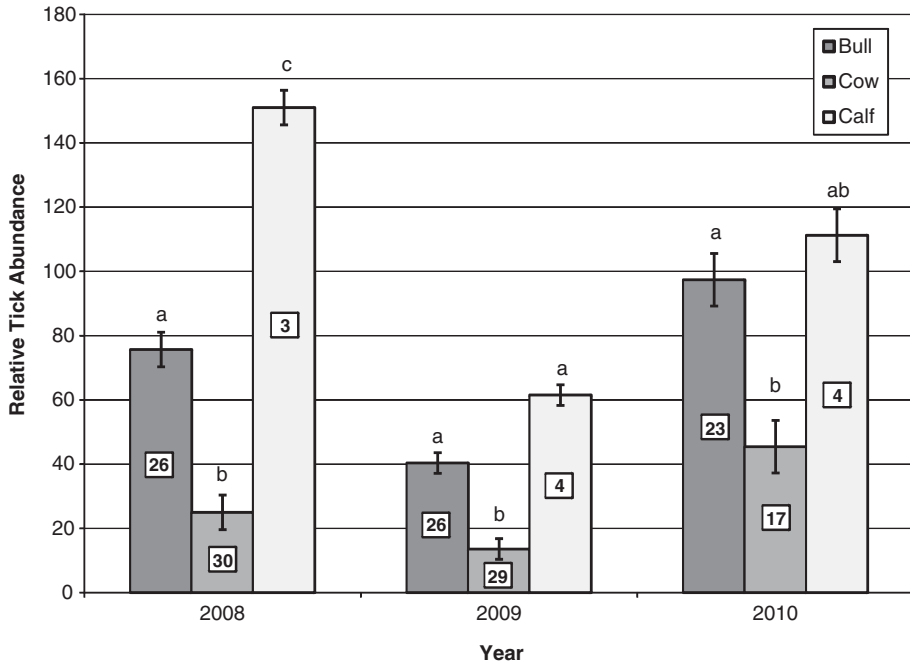


Fig. 4. Mean (\pm SE) number of winter ticks counted on harvested bull, cow, and calf moose in northern New Hampshire; data are for all sample regions combined, 2008–2010. Means are for all 4 areas of the hide and all transects combined. Numbers in columns represent sample sizes. Bars with unlike letters indicate significant differences within sample year.

Combined regional and yearly data yielded similar r^2 values for each area of the hide and all areas combined ($r^2 \approx 0.80$).

Roadside Surveys of Tick-Induced Hair-Loss on Moose

A total of 256 and 222 moose were surveyed in the 3 sample regions during spring 2009 and 2010, respectively: 86 and 72 in CT Lakes, 96 and 77 in the North, and 74 and 73 in the White Mountains. Moose in each hair-loss category were observed each year. In 2009 the CT Lakes had the highest HLI (3.23), the North was 11% lower (2.91), and the White Mountain region was 2.35 or 24% lower. In 2010, HLI values were lower in every region; the North region had the highest HLI (2.79), the CT Lakes was 14% lower (2.44), and the White Mountain region was 2.25 or 8% lower (Table 1).

Two single-day surveys were conducted on 12 and 24 May, 2010; however, only the North region produced enough sightings to

Table 1. Hair-loss severity index (HLI) values for 3 sample regions and bull, cow, and calf moose in northern, New Hampshire, 2009 and 2010. Single-day survey results for each region are included in parentheses (5/12/10 and 5/24/10). Bull, cow, and calf data were regionally combined by sample year.

Region/ Moose	2009	n	2010	n
CT Lakes	3.23	86	2.44 (2.00, 2.00)	72 (8, 3)
North	2.91	96	2.79 (2.67, 2.17)	77 (51, 23)
White Mountain	2.35	74	2.25 (2.22, 2.38)	73 (9, 8)
Combined	2.86	256	2.50	222
Bull	3.07	90	2.65	83
Cow	2.70	111	2.45	103
Calf	2.75	36	2.29	35

make a single-day survey plausible. A total of 51 and 23 moose with corresponding HLIs of 2.67 and 2.17 were observed on 12 and 24 May, values 4% and 29% lower than the regional survey. The other regions had <10 moose observations each day. The HLI of bulls, cows, and calves ranged from 2.70–3.07 in 2009 and 2.29–2.65 in 2010, and varied little between sex/age of moose (2–16%); HLI of bulls was always highest (Table 1).

Calf:cow ratios calculated during spring hair-loss surveys were mid-range of the fall moose hunter and deer hunter surveys, except in the White Mountains region in 2010 when it was lower than both surveys. There was little variation in calf:cow ratios among regions and between years; ratios ranged from 0.21–0.34 from moose hunter surveys, 0.33–0.41 from deer hunter surveys, and 0.30–0.43 from hair-loss surveys. The ratio from the single-day survey in the North region (0.38 both days) was mid-range of the moose and deer hunter surveys (Table 1). No evidence of a winter tick epizootic or major calf mortality existed either year.

DISCUSSION

Flagging for Tick Larvae

Abundance of tick larvae was correlated with regional moose density both years, which was consistent with trends identified in previous studies. In Elk Island National Park the average number of ticks on moose increased as moose numbers increased, with a 1-year lag; also, many documented large die-offs of moose in the Park occurred at peak moose density. Although it is tempting to relate high tick densities with moose die-offs, similar tick densities occurred in years with and without die-offs in the Park (Samuel 2004, 2007). Clearly the relationship is not exact, and direct comparison of estimates between disparate geographic regions may be unwarranted as other factors,

such as weather, likely play a role (DelGuidice et al. 1997, Samuel 2007).

The high variability in tick abundance in clear-cuts likely influenced the lack of statistical differences among regions and between years. Regional means were highly influenced by a few cuts with high abundance of ticks, and the high variability among clear-cuts suggests that winter ticks are not evenly distributed even within this preferred habitat type of moose. Certain clear-cuts in each region had abundance 2–7X higher than the regional mean both years; this distribution pattern may explain why individual hair-loss varies annually, and certain moose have severe hair-loss in years of overall light infestation and vice versa. Conversely, local sites with high moose and tick density may explain, in part, why epizootics usually occur across wide geographic ranges that encompass variable moose population densities.

A benefit of this sampling method is that it can extend through the entire questing period, which usually occurs from September until winter conditions kill remaining unattached larvae (usually November–December) (Drew and Samuel 1985, Samuel 2004); questing usually stops at <0° C (Samuel and Welch 1991). Because our sampling occurred from early-mid September through the first substantial snowfall, it should be representative of the relative abundance of ticks. However, because temperature and snow condition varied considerably among the adjacent study regions, tick abundance from flagging alone would not necessarily reflect regional tick abundance on moose. However, it may be possible to detect annual regional trends in tick abundance because tick numbers declined in each region from 2008 to 2009. The data also suggest that moose density influences tick abundance because relative tick density was correlated with regional moose density both years.

The flagging technique is probably not practical to index tick abundance because it is extremely labor intensive and costly. Sampling occurred for ~3 months and across a wide geographic range. Two people sampled a clear-cut in ~2 h and each needed to be visited multiple times; workdays averaged 8–10 h and counts of larvae at a later date on each flannel required 10 min→1 h depending on the number of ticks. The relative length of the questing period is probably most easily estimated by tracking ambient temperature and snow cover, and assuming that an extended warm fall will lengthen the questing period and tick abundance.

Tick Abundance on Harvested Moose

Tick abundance measured directly on harvested moose was highest in the North and lowest in the White Mountains region each year; conversely, flagging (sampling for larvae) measurements were correlated with regional moose density. Tick abundance was higher on calves than bulls and cows each year. Drew and Samuel (1985) suggested that bulls may have the highest absolute numbers of ticks due to their size and increased activity during the rut; however, calves have proportionally more ticks (per area) due to their smaller body size (Samuel and Barker 1979, Samuel 2004).

There was a strong relationship between transect counts and total counts from hide digestions, and the strongest relationships occurred when data from all areas of the hide were combined ($r^2 = 0.80$). Sine et al. (2009) also found strong relationships ($r^2 = 0.88$) when combining hide samples and concluded that the total number of ticks counted on all transects was the best predictor of tick density on moose. Due to the strong relationship between transect and total counts in both studies, we suggest that transect counts (easy and efficient) should suffice for use as an index of relative tick abundance on harvested moose. Average time to count

the 4 areas of hide was ~5 min with a separate counter and recorder, and about twice as long if done alone (same as Sine et al. 2009). Further, some hunters were unwilling to donate hide samples and laboratory work was tedious and labor intensive; hide samples took ~2 h to digest and counting tick exoskeletons varied from a few minutes to hours.

The transect method identified differences between regions and years, but did not indicate a positive correlation with moose density as did the flagging method. Because the moose harvest in New Hampshire occurs in mid-October, this method may not translate directly to tick load and/or related moose mortality if moose disproportionately acquire ticks in late fall. Aggregations of winter tick larvae can survive into November (Drew and Samuel 1985), and tick larvae were collected into December during flagging. However, if the timing of the hunting season remains constant, a useful index of relative tick abundance should be evident with a few additional years of data. Further, the highest tick abundance measured in fall 2010 preceded an epizootic in 2011 throughout the northeastern United States (pers. comm., L. Kantar and K. Rines, Maine Inland Wildlife and Fisheries and NHFG, respectively).

Roadside Surveys of Tick-Induced Hair-Loss on Moose

The HLI values were correlated positively with regional moose density in 2009, as was the flagging method; however, although HLI values declined in each region in 2010, the North region had the highest HLI, the same pattern as occurred with tick abundance on harvested moose. Overall, all methods indicated a reduction in tick numbers from the first to second year of the study (2008–2009: flagging and harvested moose, and 2009–2010: hair-loss) with combined data from all regions, suggesting that singly,

none is sensitive enough to detect potential differences in tick abundance among regions; however, any would probably detect large annual change in relative tick abundance.

Hair-loss surveys conducted in Algonquin Provincial Park, Ontario since 1984 (Steinberg 2008) had HLI values ranging from 1.18–3.48; HLI's ≥ 2.50 were associated with mortality events in 1992 and 1999. However, no epizootic occurred in 1988, 2000, or 2006 with similar values. The HLI values in this study were 2.20–3.23 with the majority > 2.5 , but no major mortality event was evident. However, direct comparison with HLI values in New Hampshire are probably unwarranted because helicopter surveys are usually conducted in March in Algonquin Park (due to snow cover), whereas surveys occurred in April–May in New Hampshire when hair-loss is more evident (McLaughlin and Addison 1986).

Bulls had the highest HLI both years, suggesting that rutting activity of bulls during the fall questing season contributes to their tick load (Drew and Samuel 1985, Samuel 2004). However, there was little variation overall (2–16%) in the HLI of bulls, cows, and calves indicating that sex/age of moose might have little influence on survey results. A minimum of 50 moose is considered an adequate sample in Algonquin Provincial Park (Steinberg 2008), and this sample size was realized in a single day survey on 12 May in the North region ($n = 51$). The HLI (2.67) was similar (4% lower) to that of the weekly survey (2.79), suggesting that a single-day survey should suffice given adequate sample size.

In New Hampshire surveys should be conducted as multiple, morning surveys, preferably condensed within a 5-day period (1 May–15 May), that are focused on the most commonly used roadside saltlicks in the highest moose density regions; the

survey would be complete with ≥ 50 individual moose. Routes within a region should be separated to ensure that the same moose is not observed at different licks by multiple observers (or use a single observer). This would reduce the duration of surveys, distance traveled, and eliminate repeat sightings. Surveys should also be conducted on cool mornings with little precipitation to enhance sightings.

Because calves are likely most impacted by winter ticks and is the cohort most susceptible to mortality, estimates of fall and spring calf:cow ratios should indicate substantial mortality events that reduce the proportion of calves in the population. Calf:cow ratios calculated from fall hunter surveys and spring hair-loss surveys were reasonably similar, and calf:cow ratios during the single-day survey in the North region ($n = 51$) were similar to those in the weekly survey. Low sample size may be problematic for calculating such ratios in spring, and the reliability and sensitivity to detect such change is unknown because no evident die-off occurred.

Conclusions

Although moose density and tick abundance were generally related in New Hampshire, weather plays a strong role in the abundance and distribution of winter ticks (Samuel and Welch 1991, Samuel 2007). Regional weather differences that impact ticks at different life stages likely influenced regional tick abundance regardless of moose density. Because major moose die-offs are usually concurrent and widespread geographically (Samuel 2004), tracking regional differences in New Hampshire may not be as important as obtaining adequate tick abundance samples from harvested moose and at least one regional sample of 50 moose from roadside hair-loss surveys.

The combination of fall tick counts on harvested moose and spring hair-loss surveys

should prove useful in indexing winter tick abundance in northern New Hampshire. Both methods are time and cost-effective and capable of indicating annual change in relative tick abundance. Check station counts provide an indication of transmission during the questing period; however, if weather conditions were to extend the questing period into December, check station counts may no longer be representative of actual tick loads. Hair-loss surveys should help identify high tick abundance in late winter-spring caused by an extended questing period, and calf:cow ratios from the surveys could detect years of high calf mortality. Combined use and comparison of these methods will increase confidence in their index value; of particular future interest is an ability to identify threshold values associated with major moose die-offs.

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