USING SNOW URINE SAMPLES TO ASSESS THE IMPACT OF WINTER TICKS ON MOOSE CALF CONDITION AND SURVIVAL

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ABSTRACT: Snow urine samples collected in northern New Hampshire, USA were used to measure urea nitrogen (UN) and creatinine (C) content to develop ratios for tracking the nutritional restriction of individual moose (*Alces alces*) through winter (2014–2017), inclusive of the adult winter tick (*Dermacentor albipictus*) engorgement period. Samples (n = 215) were collected from 55 moose (38 calves, 17 cows) on a twice monthly schedule from late January through snowmelt or calf mortality (March – early April). Early winter UN:C ratios from cows, surviving calves, and calves that ultimately died from infestation of winter ticks were similar and reflected a normal winter diet low in protein. A heightened UN:C ratio (> 3.5 mg/dL) was measured in March which aligned with peak feeding by adult winter ticks, and presumably reflected accelerated protein deficit associated with blood loss. This increase was not observed population-wide despite shared habitat, occurring only in calves with mortal weight loss and anemia associated with heavy winter tick infestation. Measurement of UN:C ratios from snow urine samples proved an effective method to measure the temporal impact of winter tick infestation, and March samples can support other metrics used to estimate calf mortality.

ALCES VOL. 55: 13–21 (2019)

Key words: creatinine, epizootic, New Hampshire, moose, nutritional restriction, snow urine, urea nitrogen, winter ticks

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New Hampshire has experienced at least 5 winter tick (*Dermacentor albipictus*) epizootics (> 50% calf mortality) in the last decade, an unprecedented rate of occurrence (Musante et al. 2010, Bergeron et al. 2013, Jones et al. 2019). Concurrent with this heightened frequency of epizootics, New Hampshire's moose (*Alces alces*) population has declined ~45% over the past 15 years (NHFG 2015). The cause of this decline in northern New Hampshire is the negative influence of winter ticks on calf survival which can be < 30% annually, and associated lower productivity of yearling and adult cows (Musante et al. 2010, Jones et al. 2017, 2019). The fate of moose calves (8–12 months old) during an epizootic is determined by their relative condition (i.e., body weight, fat stores) and the severity of tick infestation as it relates to metabolic impacts, including blood loss, protein deficiency, and subsequent weight loss (Samuel 2004, Musante et al. 2007, Ellingwood 2018). As such, monitoring the condition of calves through winter and spring should provide insight into the relative and temporal influence of tick loads on metabolic imbalance, survival, and productivity. Measurement of urea nitrogen from urine samples in snow serves as an informative measure of individual condition, reflecting muscle tissue catabolism in animals with an otherwise low-protein diet (Seal et al.

1972, Kirkpatrick et al. 1975, Bahnak et al. 1979). Radio-marked calves provide for a unique opportunity to monitor and assess specific physiological parameters from individuals with known fate through the peak mortality period associated with winter tick parasitism.

STUDY AREA

The study area (Berlin) is located within Coos County and includes sections of Wildlife Management Units (WMU) B, C1, and C2 in the towns of Berlin, Milan, Dummer, Success, Cambridge, Millsfield, Stark, and Second College Grant (Fig. 1).

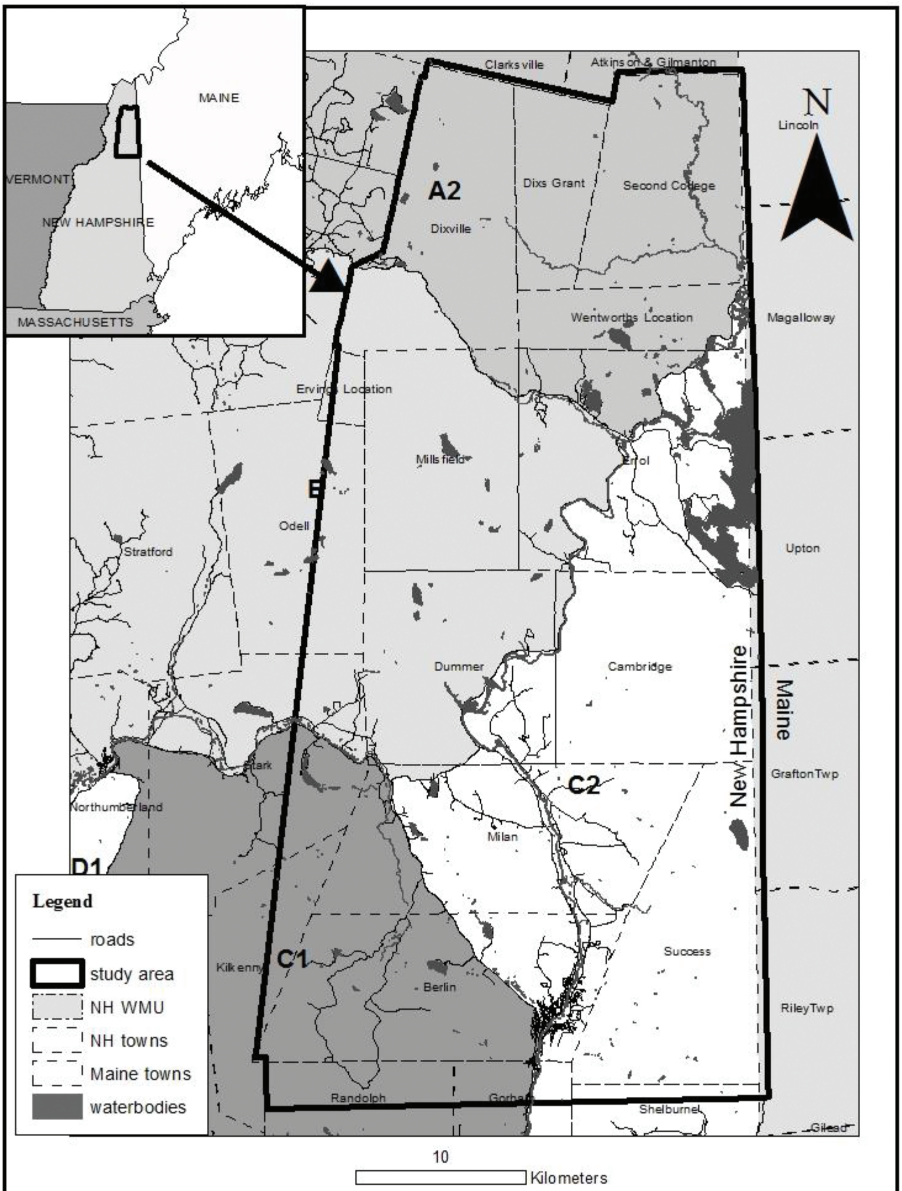

Fig.1. Location of the study area in Wildlife Management Units A2, B, C1, and C2 in Coos County, New Hampshire, USA.

The landscape is bisected by the Androscoggin River and is relatively mountainous, bordered to the west by the Kilkenny Range and the south by the Mahoosuc Range. Landcover is predominately commercial forest in which deciduous areas are dominated by yellow (*Betula alleghaniensis*) and paper birch (*B. papyrifera*), American beech (*Fagus grandifolia*), and sugar maple (*Acer saccharum*), with softwood stands characterized by black spruce (*Picea mariana*), red spruce (*P. rubens*), balsam fir (*Abies balsamea*), and white cedar (*Thuja occidentalis*) (DeGraaf et al. 1992). Logging operations remove 1–3% of timber annually, and optimal moose habitat (4–16 year-old growth) increased 2.5X between 2001 and 2015 to equal > 17% of forest cover (Dunfey-Ball 2017). Habitat quality is considered good and not a limiting factor to the local moose population (Bergeron et al. 2011, Dunfey-Ball 2017). The average date of first snowfall is 14 November, with permanent snow cover typically in December (Dunfey-Ball 2017). This site was the location of a comprehensive study of moose population dynamics in 2001–2005 when density was estimated as ~0.8 moose/km^2 (Musante et al. 2010). The most recent population estimate is ~0.6 moose/km^2, and from 2014–2018, > 200 moose were fit with radio-collars as part of a larger study. Winter tick-related calf mortality was 62%, 74%, 77%, and 30% in 2014, 2015, 2016, and 2017, respectively (Jones et al. 2019, P. J. Pekins, unpublished data).

METHODS

Capture and monitoring

Calves (~8 months old) were captured in early January 2014 – 2017 via net-gunning and aerial darting techniques (Aero Tech Inc., Clovis, New Mexico, USA in 2014 and 2015; Native Range Capture Services, Elko, Nevada, USA in 2016 and 2017). Moose were fitted with either VHF (n = 76; M2610B, Advanced Telemetry Systems, Isanti, Minnesota, USA; Mod-600, Telonics, Mesa, Arizona, USA) or GPS radio-collars (n = 104; GPS Plus Vertex Survey Collar, Vectronic Aerospace GmbH, Berlin, Germany). The tick load of each moose was measured by summing the number of ticks on 4, 10 cm transects on both the shoulder and rump (Sine et al. 2009, Bergeron and Pekins 2014). The sum of those 8 transects is used as an index to compare relative tick loads between individuals and across years.

The VHF radio-collars transmitted at 55 pulses/min (ppm) while active, and switched to 110 ppm after 4 h without movement, signifying a mortality event. The GPS radio-collars transmitted 2 location fixes/day (00:00 and 12:00 hr EST) which were viewed and downloaded via Vectronics Aerospace software (GPS PLUS X V10.4), and also had a VHF beacon transmitting at the same pulse rates as the VHF radio-collars. These radio-collars switched to mortality mode and sent a mortality alert via email after 5 h of inactivity. All calves were necropsied in the field following standard procedures (Mason and Madden 2007, Munson 2015), including an external examination, body weight measurement, and collection of tissue samples for subsequent examination at the New Hampshire Veterinary Diagnostics Lab (Durham, New Hampshire) to determine cause of death (Jones et al. 2019).

Urea nitrogen:creatinine sampling

Snow urine samples were used to track the nutritional restriction of individual animals through the winter, inclusive of the adult tick engorgement period. Samples were collected from radio-marked calves and accompanying unmarked adult cows presumed to be the mothers. An effort was made to collect a sample from each individual every 2 weeks beginning in late January and extending through snowmelt or mortality (March – early April); the goal was to collect 3–6 temporal samples per individual. Samples were collected within 24 h of urination by locating the bedding site and/or tracks in the snow at coordinates transmitted by the GPS radio-collar at 00:00 hr that day; tracks were followed until a sample was identified. Moose with VHF radio-collars were located using ground telemetry techniques (Mech 1983) and back-tracked to collect urine samples. In cases where an adult accompanied the calf, samples were distinguished between the pair by the size of tracks and bed nearest to the sample. Consistent with the methods used by DelGiudice et al. (1988), the most concentrated portion was collected in plastic bags using rubber gloves to avoid contamination. Samples were subsequently thawed at room temperature and aliquoted into 2 mL cryovials. These aliquots were stored frozen until submission to BiovetUSA (Burnsville, Minnesota) for measurement of urea nitrogen (UN) and urinary creatinine (C) content (mg/dL). These data were expressed as a ratio (UN:C) to correct for the dilution of each sample by snow (DelGiudice et al. 1988); C is proportional to muscle mass and remains near constant in individuals over a given day (DelGiudice and Seal 1988). Analysis included those individuals that were sampled most consistently, while attempting to achieve near equal representation of surviving and dead calves.

The UN:C ratio of urine samples were $\log_e$ transformed to stabilize the variance in the dataset prior to analysis (DelGiudice et al. 1989). Samples were initially grouped by fate (mortality or survivor), and samples within each group were pooled at 2-week intervals (each individual was sampled every other week). A two-way ANOVA was used to examine differences in the mean UN:C ratio of samples collected from cows, surviving calves, and dead calves across collection intervals. Tukey's range (HSD) test was used to make post-hoc comparisons between multiple collection periods.

RESULTS

Winter mortality of radio-marked calves in 2014 – 2016 averaged 71%, dropping to 30% in 2017; the leading cause of death (> 90%) was winter tick parasitism (Ellingwood 2018, Jones et al. 2019). A total of 158 snow urine samples were collected from 38 radio-marked calves (23 winter mortalities and 15 survivors) in the winters of 2014–2017. Samples (n = 57) were also collected from 17 unmarked adult cows accompanying a portion of these marked calves; all adult cows were presumed to survive through winter. Maximum annual snow depth during the collection periods ranged from 17.8 – 71.1 cm.

From ~15 January – 14 February, there were no statistical differences between UN:C ratios of unmarked cows, surviving calves, and calves that died ($P > 0.05$). From ~ 15 Feb – 15 March, the mean UN:C ratio of calves that died and those that survived diverged significantly ($P < 0.05$), whereas the UN:C ratio of cows and surviving calves remained similar. The UN:C ratios of unmarked adult cows stayed stable throughout the sampling season ($\bar{x} = 2.35 \pm 0.85$), with individual samples ranging from 0.8–4.54 with no difference detected between collection intervals ($P > 0.05$; Fig. 2). The average UN:C ratio of calves that died increased through the second week of March, peaking at 4.68 ± 2.93; the ratio of survivors was lower and relatively stable during this same time period ($\bar{x} = 2.43 \pm 0.74$; Table 1). In the weeks following this spike in UN:C ratios, the average ratio of calves that died never returned to levels < 3.5, and mortality occurred 1–5 weeks after their UN:C ratio peaked ($\bar{x} = 3$ weeks).

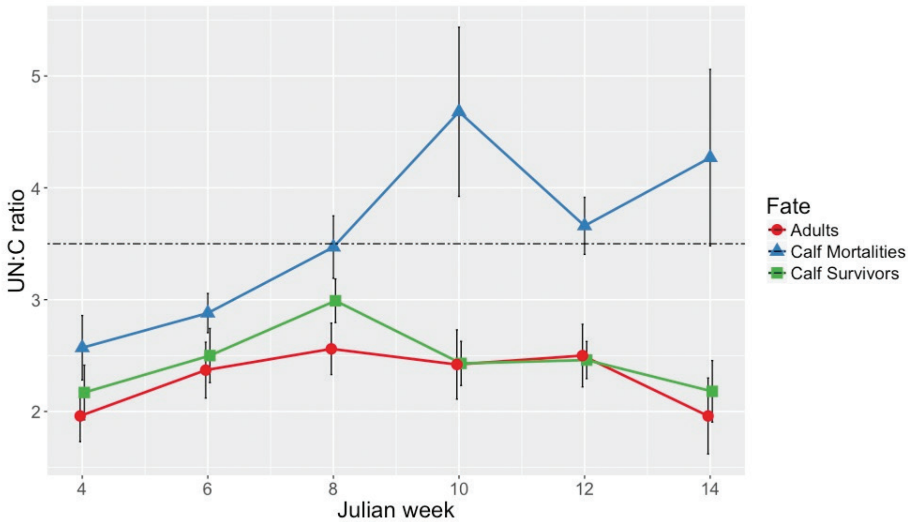

Fig. 2. Mean (± SE) UN:C ratios in snow urine samples collected from radio-marked calves and unmarked adult cow moose in northern New Hampshire (January-April, 2014–2017). Dotted line at UN:C ratio = 3.5 represents the survival threshold described by DelGiudice (1995).

Table 1. Summary of UN:C ratios of snow-urine samples (n) from moose in 2014–2017, New Hampshire, USA. Original UN:C data are presented here with statistical comparisons made after data were $\log_e$ transformed. Dead calves had significantly higher (*P* < 0.05; *) UN:C ratios from late February onward.

Collection interval	Surviving calves			Dead calves			Unmarked adult cows		
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD
15–31 Jan.	12	2.17	0.85	13	2.57	1.04	7	1.96	0.60
1–14 Feb.	11	2.50	0.80	19	2.88	0.97	12	2.37	0.87
15–28 Feb	15	2.99	0.75	18	3.58*	1.19	11	2.56	0.77
1–14 Mar.	14	2.43	0.74	15	4.68*	2.93	9	2.42	0.93
15–31 Mar.	10	2.47	0.53	12	3.66*	0.88	13	2.50	1.01
1–14 Apr.	10	2.18	0.87	9	4.27*	2.37	5	1.96	0.77

Spring snowmelt prevented sample collection from 14 calves within 2 weeks of death.

DISCUSSION

Moose are on a negative nutritional plane from winter through early spring, although winter mortality from malnutrition is considered rare in the northeastern United States. Adult survival rates in late winter average 97% and starvation has not been identified as a source of mortality regionally (Musante et al. 2010, Jones et al. 2019). A low level of undernutrition during winter is typical and reversible, as fat stores are used to supplement the reduced nutritional quality of winter browse (DelGiudice and Seal 1988, DelGiudice and Moen 1997, Schwartz and Renecker 2007). Winter diets have limited digestible protein which is reflected by low UN:C ratios in moose urine, and as fat stores deplete, moose increase catabolism of muscle protein (rich in nitrogen) to meet protein and energy requirements, causing an abrupt spike in UN:C ratios (DelGiudice et al. 1987, DelGiudice and Seal 1988). Ratios > 3.5 represent serious deterioration in body condition with estimated losses of 0.5–0.8 kg/day of lean body (muscle) mass for a 400 kg moose (DelGuidice et al. 1997). For an average (174 kg) calf expressing similar UN:C ratios, this would equate to 0.2–0.3 kg/day or ~5% weight loss in 30 days.

Low UN:C levels (< 3.5) were measured in this study from January through late-February each year, suggesting that all cows and calves were in an early phase of undernutrition. The spike in UN:C ratios measured in calves that died is symptomatic of animals experiencing prolonged undernutrition and catabolizing muscle. Similar effects have been measured in white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*), and other moose populations due to limited forage diversity, availability, and quality associated with marginal habitat and deep snow (DelGiudice et al. 1989, 1991a, 1991b). All dead calves expressed signs of anemia and emaciation including severe weight loss (23 ± 7%) in 3.5 months (Ellingwood 2018). This degree of weight loss is comparable to that measured in white-tailed deer experiencing prolonged and irreversible levels of malnutrition (DelGiudice and Seal 1988).

In contrast to the abovementioned examples, the impact of undernutrition in this study was not evident population-wide or associated with habitat or winter severity. Calves that died experienced a UN:C spike in March, whereas surviving calves and adult cows did not. Surviving calves and adults shared the same habitat as the dead calves, and forage availability in the study area is not considered limiting (Bergeron et al. 2011, Dunfey-Ball 2017). Limited mobility and increased mortality due to snow depth for moose occurs at >90 cm (Coady 1974); however, in the winters of 2014–2017, average snow depth was ≤ 71 cm in northern New Hampshire and the highest rate of calf mortality occurred in the year of lowest maximum snow depth (Jones et al. 2019).

The timing of the spike in dead calves aligns with the peak feeding period of adult winter ticks in mid- to late March (Samuel 2004). Arguably, calves were experiencing an increasing protein and energy deficit due to the physiological requirement to replenish blood (protein) loss associated with feeding by winter ticks. Tick load estimates derived from half-hide counts of dead calves averaged 47,000 (Jones et al. 2019), and compensation for blood loss associated with this infestation level requires 50 to >100% of the daily protein requirement of a calf during the peak 2-week feeding period of adult winter ticks (Musante et al. 2007). Metabolically, this depletion of muscle mass (protein) mimics that of a starving animal, producing a similar response in the UN:C ratios. For example, DelGiudice and Seal (1988) identified UN:C ratios ranging from 4 - >23 mg/dL in urine samples collected from white-tailed deer experiencing prolonged undernutrition. The average UN:C ratio peaked at 4.68 for dead calves; however, given the average period (20 ± 13 days) between the date of the last sample collection and mortality, our data do not reflect the higher ratios that occur closer to death.

The tick load measured at January capture was the primary determinant of calf fate, as tick load and probability of survival were inversely related. For calves with low and moderate tick counts, body weight has some counter-balancing effect on survival, with heavier calves (> 174 kg) expressing heightened resistance to mortality (Ellingwood 2018). This influence was also demonstrated in higher survival of heavier calves in northern Maine with similar tick loads. The body weight of 73% of calves captured in New Hampshire was less than the average weight (190 kg) in northern Maine where winter tick epizootics remain uncommon (Ellingwood 2018; L. Kantar, Maine Department of Inland Fisheries and Wildlife, unpublished data). Given the high proportion (17.5%) of optimal habitat (4–16 year old regenerating growth) in the region (Dunfey-Ball 2017), these low weights presumably reflect the carry-over effects of compromised reproductive cows impacted by high annual tick loads (Musante et al. 2010, Jones et al. 2017).

Temporal monitoring of UN:C ratios identified the occurrence and timing of nutritional restriction of calves that ultimately died from blood loss to winter ticks. While all moose exhibited some degree of undernutrition, the spike observed in the UN:C ratio of dead calves was related directly to the feeding period of adult female winter ticks. In more southern moose populations where winter ticks are of most concern, UN:C ratios from snow urine samples could be used to assess calf condition, identify a potential epizootic (DelGiudice et al. 1997), and predict mortality rate in the population. Collection and analysis of snow urine samples from calves in the second and third week in March should be adequate to identify the proportion with UN:C ratio > 3.5 and provide a reasonable estimate of the seasonal calf mortality rate. The ability to measure this metric provides critical recruitment information and is a useful proxy for productivity, both necessary to develop effective management strategies.

ACKNOWLEDGEMENTS

Funding for this project was provided through Wildlife Restoration Program Grant No. F13AF01123 (NH W-104-R-1) to N.H. Fish and Game Department from the U.S. Fish and Wildlife Service, Division of Wildlife and Sport Fish Restoration, and Safari Club International Foundation. This research was made possible through the access granted by property owners including American Forest Management, the Conservation Fund, Plum Creek, T. R. Dillon, and Wagner Forest Management Ltd. The efforts of field technicians J. DeBow and T. Soucy were critical for the collection of these data.

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