Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-central Alaska

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Abstract. Recent studies suggest that the growth and fecundity of northern ungulates may be coupled to their summer nutrition. Here, we compare summer dietary nitrogen availability of the five major browse plants (comprising ~79% of the diet) of moose (*Alces alces*) in Denali National Park and Nelchina Basin, Alaska, USA. In recent years the productivity of Denali moose has been significantly higher than that of Nelchina moose, prompting this comparison. We examined the phenological progression of leaf nitrogen concentration, tannin–protein precipitation capacity, and digestible protein over three summers in both regions. We then modeled the potential nutritional consequences for a cow moose consuming representative diets on each range, predicting both net protein intake (NPI) and lean body mass accumulation each year. We found that leaf nitrogen and digestible protein decreased, while tannin–protein precipitation capacity increased throughout the summer for all forages. There was 23% more digestible protein in Denali leaves than Nelchina leaves on average, and this difference was significant in all three years. Tannins accounted for a large (mean = 46%) reduction in protein availability, suggesting a key role of these secondary compounds in the nitrogen balance of moose in these regions. Finally, our NPI model predicted that Denali cows were in positive protein balance 17 days longer than Nelchina cows and accumulated 18 kg more lean body mass over the summer, on average. We conclude that summer dietary nitrogen availability may act as a nutritional constraint on moose and suggest that more emphasis be placed on elucidating its role in population dynamics and conservation of northern ungulates.

Key words: Alces alces; bottom-up regulation; Denali National Park, Alaska; modeling; Nelchina Basin, Alaska; nitrogen; nutrition; protein precipitation; tannin; top-down limitation; ungulate; willow.

Introduction

Northern ungulates undergo pronounced seasonal fluctuations in body reserves, with peak body masses generally reached in the fall (Mautz 1978, Adamczewski et al. 1987, Schwartz et al. 1987*a*). An important life history consequence of this seasonal gain is that autumn body mass is often linked to an individual's reproductive success (Cameron et al. 1993, Schwartz and Hundertmark 1993, Cameron and Ver Hoef 1994; but see, e.g., Barrett 1982). Although winter nutrition has generally been considered limiting to productivity (e.g., Wallmo et al. 1977, Adamczewski et al. 1993, Mysterud and Ostbye 2006), a growing body of evidence (Hjeljord and Histol 1999, Cook et al. 2004, Stewart et al. 2005, Herfindal et al. 2006) supports the theory of Klein (1970) that foraging conditions in the spring and summer have the greatest influence on growth and therefore may largely influence the fall body mass and subsequent reproductive potential of many large herbivores.

Diet quality is an important determinant of animal productivity because of its multiplier effect on body condition (White 1983). In general, more digestible foods can be consumed in greater quantities because both passage and digestion rates are enhanced (Robbins 1993). Although digestible energy has typically been the focus of previous nutritional studies (Parker et al. 1999, Cook et al. 2001, Cook et al. 2004), digestible protein may be underappreciated in the boreal environment. Nitrogen is often a limiting nutrient to plant growth in this ecoregion (Bryant et al. 1983, Van Cleve et al. 1983, Coley et al. 1985, Schimel et al. 1996) and this limitation to plants may cascade in its limitation to herbivores as well (White 1993). Of particular importance to browsing herbivores, low nitrogen concentration in plants is exacerbated by the presence of tannins, which quantitatively reduce protein availability (Robbins et al. 1987).

Significant gaps remain in our understanding of large-herbivore seasonal protein balance, but it is clear that adequate protein reserves are critical to the spring reproductive success of females. For example, in caribou

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(*Rangifer tarandus*), endogenous maternal protein is probably the primary source of fetal protein accretion in late winter (Parker et al. 2005), coinciding with the last trimester of gestation when 80% of fetal mass is deposited (Robbins and Robbins 1979, Oftedal 1985). Concurrently, calf body mass is correlated with maternal body protein reserves (Allaye-Chan 1991) and body mass at birth is often correlated with survival (Clutton-Brock et al. 1987, Clutton-Brock and Albon 1989, Fairbanks 1993). Summer protein intake is especially important for lactating females. These animals must meet the nutritional demands for growth and replenishment of body condition prior to the next breeding cycle and winter (Crete and Huot 1993, Gerhart et al. 1997), while at the same time meeting the protein demand for milk production, which is often close in value to their own maintenance requirements (White and Luick 1984, Renecker 1987, Reese and Robbins 1994). Thus, the additional demand of lactation can effectively double a cow's overall summer protein requirement.

In spite of the recognized importance of protein in the reproductive performance of herbivores, surprisingly little is known about its availability in browse during the summer and among years. Although it is commonly observed that leaf N concentration declines as summer progresses (Chapin et al. 1980, Lenart et al. 2002, Rohrs-Richey and Mulder 2007), to our knowledge no studies have investigated the potential for tannins to exacerbate this nutritional decline to browsing herbivores. Likewise, only a few studies have examined yearly variation in leaf N concentration or phenolics/tannins (Bo and Hjeljord 1991, Graglia et al. 2001), or investigated the site-specific variance in plant nutritional characteristics (Graglia et al. 2001, Brenes-Arguedas and Coley 2005), and none of these studies has examined the potential associations between plant nutritional characteristics and browsing herbivore productivity.

Here we measure and compare summer nitrogen availability of the principal forages of moose in two regions in south-central Alaska: Denali National Park and Nelchina Basin near Glenallen, Alaska, USA. These regions were chosen because the productivity of moose on the two ranges was distinctly different in the years immediately preceding this study. Moose in Denali had relatively high twinning rates (44% of adult females; see Plate 1) and the age of first parturition in females was low (24 months) (Boertje et al. 2007; L. G. Adams, *unpublished* data). In contrast, twinning rates of Nelchina moose averaged 17% (1994–2003), and age at first parturition was >36 months (Testa 2004, Boertje et al. 2007). To estimate nitrogen availability and protein balance of moose on these two ranges, we first determined the relative leaf N concentration, tannin–protein precipitation capacity (PPC), and digestible protein (DP) of five primary browse species over three summers between 2001 and 2004. We then combined these plant quality data with diet composition data from the literature and our own browsing observations to estimate the protein

nutritional plane of moose between regions and years. Although this approach cannot test for a causal relationship between protein availability and moose reproductive success, it (1) provides the first analysis of conditions necessary to cause protein limitation in moose, and (2) is a sufficient test for rejection of the hypothesis that protein limits moose productivity.

METHODS AND MATERIALS

Field methods

Our objective was to evaluate the nutritional quality of the foods consumed by moose in these two study areas. Because moose browse selectively, especially when forage quantity and quality are high (Vivas and Sæther 1987, Sæther and Anderson 1990), and because predation pressure may influence where ungulates forage (Edwards 1983, Creel and Christianson 2008), we chose to sample plants only where individual animals were foraging. To obtain an unbiased sample of foraging locations, we located previously radio-collared moose from the air or by ground-tracking with telemetry receivers, and recorded their locations via GPS. The area surveyed on each sampling trip to each study area was ~500 km². In most cases, 10 or more radio-collared moose were found within a study area on a given date. When fewer than five radio-collared moose were found, non-radio-collared moose sighted during telemetry flights were also utilized to identify sampling sites. We chose five sites randomly from all moose located in each region on a particular date and visited their locations for plant collections. Field sampling of the sites occurred generally within a day and always within seven days after locating the moose. We attempted to sample each region three times per summer, although wildfires in 2003 and 2004 disrupted our sampling design by preventing three radio-tracking flights.

In Denali National Park (63°43′ N, 149°5′ W), we sampled between the Savage River drainage to the west, ~2 km east of the Parks Highway to the east, and within 8 km north and south of the park road. In the Nelchina Basin (62°25′ N, 147°29′ W), we sampled within a 20 km radius of Square Lake. Sample sites in Denali and Nelchina were visited either two or three times each summer between mid-June (~1–2 weeks after leaf flush in both study sites) and early September. We sampled in Denali from 18 to 21 June, 21 to 24 July, and 20 to 22 August in 2001; 17 to 18 June and 19 to 20 August in 2003; and 23 June, 20 July, and 31 August in 2004. We sampled in Nelchina from 28 June to 1 July, 30 July to 3 August, and 2 to 4 September in 2001; 28 June and 20 August in 2003; and 24 July and 1 September in 2004. Hereafter, we refer to late June and early July as "early summer," late July and early August as "midsummer," and late August and early September as "late summer."

At each sampling site, the nearest recently browsed *Salix pulchra* plant was chosen as the focal point of browse sampling. We then sampled foliage from three randomly selected individual plants from each of the

following species, if present: *Betula nana* and four *Salix* species, *S. alaxensis*, *S. glauca*, *S. pulchra*, and *S. richardsonii*. These five species comprise the majority of moose summer diet in both study regions (Van Ballenberghe et al. 1989; W. B. Collins and D. E. Spalinger, *unpublished data*). We sampled the plants by attempting to mimic moose browsing by stripping the leaves and tips of the current annual growth from several branches per plant. All samples were frozen on dry ice immediately, and they remained frozen until they were freeze-dried in the lab. For each plant sampled, we also recorded an index of summer browse scarring severity based on a simple scale, ranging from no damage, light (1–25%), moderate (25–75%), or heavy (75–100%).

Laboratory methods

Freeze-dried leaf tissue was ground in a Wiley mill over a 20-mesh (1-mm) screen and stored in airtight containers at room temperature in dark until analysis. N concentration (percentage of dry mass) was determined by the Dumas method using either a LECO 1000 CNH analyzer (LECO, St. Joseph, Michigan, USA) or a Carlo Erba Model Na 1500 N analyzer (Carlo Erba, Milan, Italy). A subset of samples was analyzed on both machines to confirm accuracy. Apple leaves (National Institute of Standards and Technology, U.S. Department of Commerce) were used as a quality control and were run at every 10th sample. Tannin–protein precipitation capacity (PPC) was determined using the method of McArt et al. (2006). Finally, digestible protein concentration was calculated using the equation of Robbins et al. (1987), $Z = -3.87 + 0.9283X - 11.82Y$, where Z is digestible protein content as a percentage of dry matter, X is crude protein content as a percentage of dry matter ($6.25 \times$ N concentration), and Y is the protein precipitation capacity ($\mu g/\mu g$ forage dry matter).

Protein simulations

To examine the potential for N limitation in moose, we modeled the minimum N requirements of an adult moose (450-kg body mass) over summer and compared this requirement to the intake of N based on estimated intake rates and our measured estimates of N digestibility of these forages. We computed net protein intake (NPI), the date at which N balance becomes negative each summer or fall, and the estimated lean body mass gain over summer. We compared the N balance of a non-lactating female moose to those of females lactating to support either a single calf or twin calves over summer. Protein requirements for moose were calculated by summing the daily estimated excretion of endogenous urinary N (56 mg/kg$^{0.75}$ per day; Schwartz et al. 1987*b*) and metabolic fecal N (5.536 g N/kg food intake; Robbins et al. 1987), compensating for efficiency of protein metabolism of food (0.80; Robbins 1993). In addition, the data from Reese and Robbins (1994) were used to compute the N demands for lactation (again at a

conversion efficiency of 0.80). Summer dry matter intake for non-lactating cows (BM = body mass) was estimated to be 130 g/kg BM$^{0.75}$ per day (Renecker and Hudson 1985). Although intake rates for cows supporting calves have not been measured to our knowledge, we have assumed that cows supporting one calf have ~10% higher intake rates (143 g/kg BM$^{0.75}$ per day) and cows supporting two calves are assumed to have approximately 10% higher intake than these cows (156 g/kg BM$^{0.75}$ per day).

We chose to start our simulations on the average first sampling date (20 June) and end the simulation on 1 September, which coincides closely with the last dates of sampling in each region. Although we may therefore miss some protein accretion by moose in early summer between leaf-out and 20 June, our purpose here is not to attempt a simulation of the actual protein dynamics of moose on these two ranges (which requires a comprehensive assessment of moose diets throughout the entire summer), but to illustrate the effects that summer protein availability might have on nitrogen balance and body condition in the fall. Our aim, therefore, is to provide a relative nutritional comparison between regions and years and insight into the potential causes of differential reproductive success for these two important moose populations in Alaska.

Simulations were conducted on two potential diets, based on published and unpublished data on moose diets in the two study areas. The five browse species that we monitored in this study comprised 79% of the summer diet of moose in Denali (Van Ballenberghe et al. 1989), and in accordance with these data, the diet for this simulation (hereafter referred to as the "Denali diet") was composed of 58% *S. pulchra*, 16% *S. richardsonii*, 9% *S. glauca*, 9% *Betula nana*, and 8% *S. alaxensis*. In Nelchina Basin, we conducted foraging studies on tame moose during July 2006 (W. B. Collins and D. E. Spalinger, *unpublished data*). Based on this work, we estimated that these five browse species comprised >99% of the diet, and the composition of this simulation diet (hereafter referred to as the "Nelchina diet") was 48% *S. alaxensis*, 24% *S. glauca*, 13% *S. pulchra*, 8% *B. nana*, and 7% *S. richardsonii*.

Statistical analyses

We first analyzed our entire data set with a mixed-model ANOVA (JMP Version 7.0; SAS Institute 2007). The format for the model was: dependent variable = region + sample date + year + species + species × region + species × year + region × year + site (nested within region and year), where the dependent variables were leaf N concentration, protein precipitation capacity (PPC), and digestible protein (DP). Next, because we found that PPC increased and N and DP concentrations decreased in a roughly linear fashion over the summer, we compared these variables between regions, years, and species using analysis of covariance. We regarded region, year, or species as factors, sample date

TABLE 1. Comparison of protein precipitating capacity of tannins, nitrogen concentrations, and digestible protein concentrations of the principal woody browse plants of the Denali and Nelchina study areas, Alaska, USA.

Comparison	Protein precipitating capacity (mg BSA/mg DM)			Nitrogen concentration (% DM)			Digestible protein (% DM)		
	LS mean (SE)	F	Diff. (%)	LS mean (SE)	F	Diff. (%)	LS mean (SE)	F	Diff. (%)
Region		10.5**			18.2****			19.0****	
Denali	0.221^a† (0.004)			2.31^a (0.02)			6.89^a (0.15)		
Nelchina	0.254^b (0.005)		+15	2.10^b (0.02)		−9	5.33^b (0.17)		−23
Year		5.6**			13.4****			13.3****	
2001	0.225^a‡ (0.005)			2.31^a‡ (0.02)			6.87^a‡ (0.16)		
2003	0.261^b (0.007)		+16	2.23^a (0.03)		−3	5.97^a (0.24)		−1
2004	0.237^{ab} (0.006)		+5	2.03^b (0.03)		−12	5.13^b (0.21)		−26
Date		116.9****			275.3****			281.0****	
Early summer	0.190^a (0.005)			2.75^a (0.03)			9.84^a (0.20)		
Midsummer	0.233^b (0.005)		+23	2.25^b (0.03)		−18	6.41^b (0.20)		−35
Late summer	0.280^c (0.006)		+47	1.75^c (0.03)		−36	2.98^c (0.18)		−70
Species		43.2****			4.2**			13.5****	
S. alaxensis	0.194^a (0.008)		+2	2.53^a (0.04)			8.51^a (0.29)		
B. nana	0.191^a (0.005)			2.25^b (0.03)		−11	6.92^b (0.20)		−19
S. richardsonii	0.191^a (0.005)		+25	2.16^b (0.05)		−15	5.84^c (0.31)		−31
S. pulchra	0.280^b (0.005)		+47	2.17^b (0.03)		−14	5.40^c (0.19)		−37
S. glauca	0.269^b (0.007)		+41	2.14^b (0.04)		−15	5.38^c (0.24)		−37

Notes: BSA is bovine serum albumin precipitation; DM is dry matter; the two plant genera are *Salix* and *Betula*. Within each comparison group in the Diff. columns, the blank cell is the basis for comparison. For example, N concentration was 9% lower in Nelchina than Denali. Protein precipitating capacity was 16% higher in 2003 than in 2001, and 2% higher for *S. alaxensis* than for *B. nana*. Within a group of factors, rows distinguished by different lowercase letters are significantly different from each other (Tukey's hsd, $P < 0.05$). Significance of F tests (linear ANCOVA) within a factor is indicated by asterisks.

** $P < 0.01$; **** $P < 0.0001$.

† Significant region × year interaction.

‡ Significant year × species interaction.

as the covariate, and tested for interactions of sample date × region, sample date × year, or sample date × species on the dependent variables PPC, N, and DP. Because it was not possible to sample in both regions at the same time, in order to compare plant nutritional characteristics at individual sample dates (i.e., early, mid, and late summer), we used the linear ANCOVA models to predict plant nutritional characteristics at a common midpoint date for the overall data set (Table 1) or for each species, region, year, and sample date (as in Fig. 3).

RESULTS

During the three years of fieldwork encompassing this study, we sampled the vegetation in Denali on eight occasions and in Nelchina on seven occasions. Among these visits, we collected browse samples at 75 sites, and analyzed PPC, N and DP on 678 plant samples.

Protein precipitation capacity and N concentration dynamics

PPC and N concentrations of species sampled during the study varied strongly with respect to all factors, including sample date, region, year, species, and site within region and year. PPC differed most strongly among sample dates ($F_{1,2} = 116.9$, $P < 0.0001$) and species ($F_{1,4} = 43.2$, $P < 0.0001$), followed by region ($F_{1,1} = 10.5$, $P = 0.002$), and year ($F_{1,2} = 5.6$, $P = 0.005$; Table

1), whereas site explained 6% of the variation within region and year ($P < 0.05$). Similar responses were found for N concentrations of the browses sampled. N concentrations differed significantly across date ($F_{1,2} = 275.3$, $P < 0.0001$), region ($F_{1,1} = 18.2$, $P < 0.0001$), year ($F_{1,2} = 13.4$, $P < 0.0001$), and species ($F_{1,4} = 4.2$, $P = 0.0025$), whereas site explained 24% of the variation within region and year ($P < 0.05$) (Table 1).

Browse quality declined as summer progressed in both regions, with N concentrations decreasing and PPC increasing in the leaves of all species (Table 1, Figs. 1 and 2). In summers when three collections were obtained, these changes were generally linear over the season (Figs. 1 and 2, Appendix B: Fig. B1, Appendix C, Fig. C1). PPC and N concentrations were highly inversely correlated with each other, and across all species (averaged by region, year, and sample date), the correlation coefficient between the two nutritional parameters was −0.67 ($n = 67$, $r^2 = 0.45$).

We found consistent regional variation in PPC and N concentration of the five browses. On average, PPC was 15% higher and N concentration was 9% lower in Nelchina compared to Denali (Table 1). This pattern was consistent across all study years, with PPC of Nelchina browses 13% higher in 2001, 16% higher in 2003, and 13% higher in 2004, whereas N concentrations were 11%, 13%, and 8% lower each year, respectively (Appendix A: Table A1). This regional plant quality
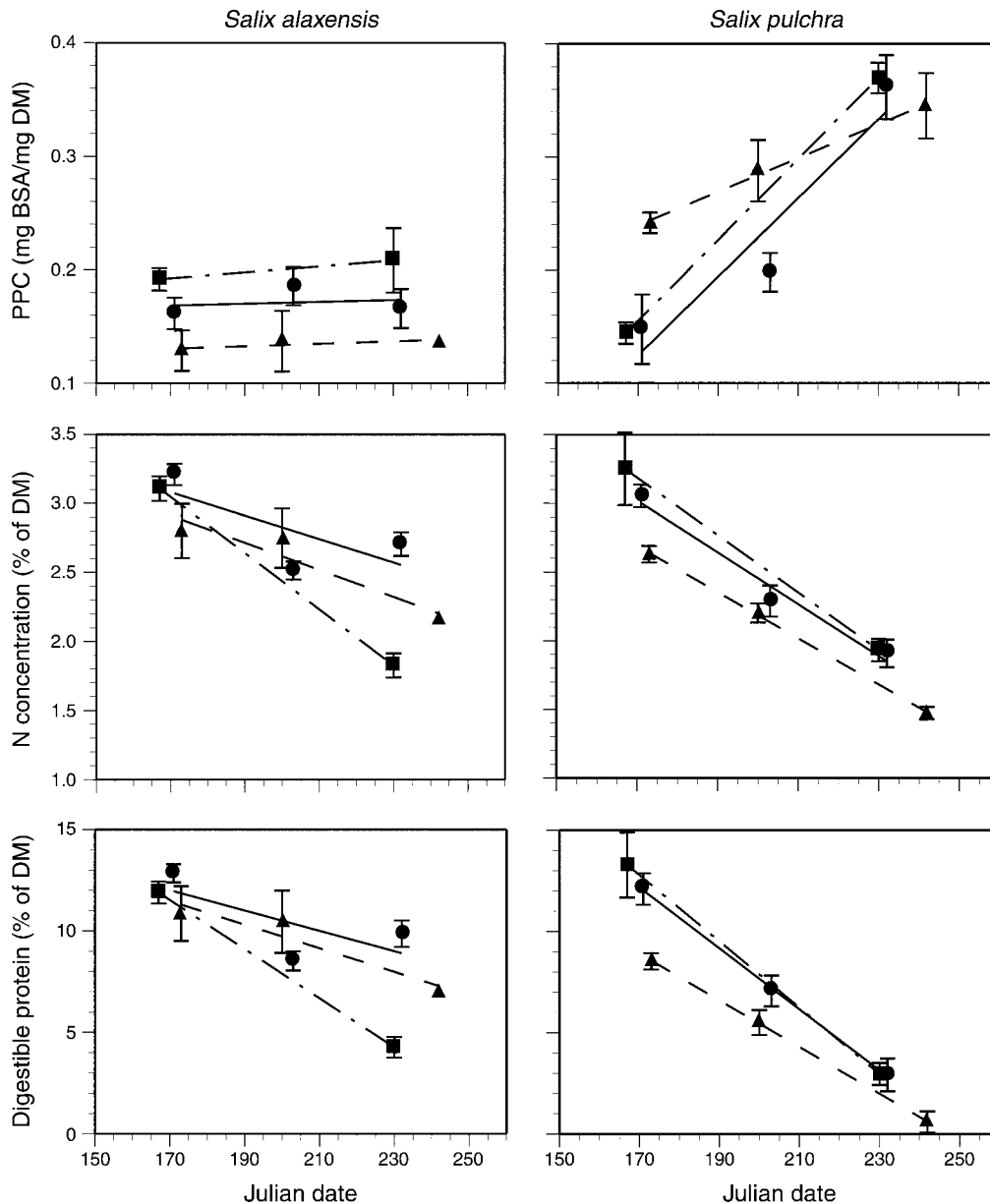
FIG. 1. The phenological progression of protein precipitation capacity (PPC), N concentration, and digestible protein concentration as a percentage of dry matter (DM) of two representative browse species in Denali National Park, Alaska, during the summer of 2001 (solid circles, solid lines), 2003 (solid squares, double dashed lines), and 2004 (solid triangles, dashed lines). Values are means ± SE. Julian day 1 is 1 January. BSA is bovine serum albumin precipitation.

pattern was consistent across all five species as well, with PPC ranging from 2% higher (*S. pulchra*) to 29% higher (*B. nana*), and N concentrations ranging from 4% lower (*S. richardsonii*) to 12% lower (*S. glauca*) in Nelchina compared to Denali (Appendix A: Table A1).

We also found significant yearly variation of PPC and N in the five browses. PPC was 16% higher in browse leaves in 2003 than in 2001, and of intermediate levels in 2004. In contrast, N concentrations were highest in 2001, averaging 2.31% of dry matter (DM), and lowest in 2004 (2.03% of DM, ~12% lower), with concentrations in

2003 being intermediate but not significantly different than N concentrations of browses in 2001 (Table 1).

In general, those species that exhibited high levels of PPC also were those with low concentrations of N. The lowest levels of PPC were found in *S. alaxensis* and *B. nana* (LS mean = 0.19 mg BSA/mg forage DM), followed by *S. richardsonii* (0.24 mg BSA/mg forage DM), and by *S. glauca* and *S. pulchra* (0.27 and 0.28 mg BSA/mg forage DM, respectively). In contrast, N concentration was significantly higher in *S. alaxensis* (2.53 % of DM) than in any of the other browses
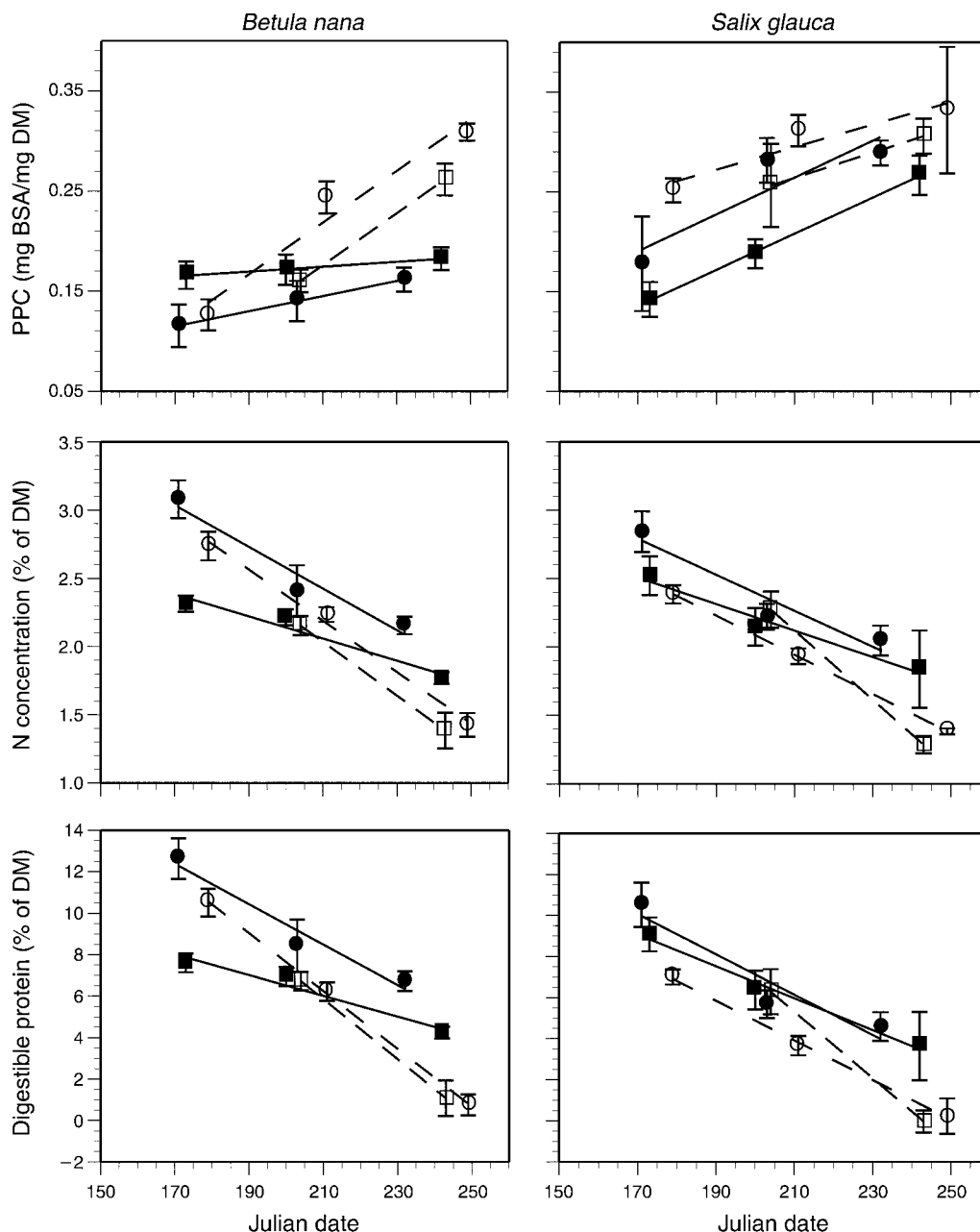
Fig. 2. The phenological progression of protein precipitation capacity (PPC), N concentration, and digestible protein concentration as a percentage of dry matter (DM) of two representative browses in Denali (solid symbols, solid lines) and Nelchina (open symbols, dashed lines) for 2001 (circles) and 2004 (squares). Values are means ± SE. Julian day 1 is 1 January. BSA is bovine serum albumin precipitation.

(ranging from 2.25% of DM in *B. nana* to 2.14% in *S. glauca*).

Digestible protein dynamics

The inverse relationship between PPC and N concentration in browse leaves, and the differences in their values between regions, years, sample date, and species resulted in highly significant differences in digestible protein (DP) among all of these factors (Table 1). As

noted for both PPC and N, DP in browse leaves was most affected by sample date. Early-summer leaf DP was 35% higher than in midsummer leaves, and ~70% higher than in late-summer leaves ($F_{1,2} = 281.0$, $P < 0.0001$; Table 1, Figs. 1 and 2). The decline in N and the increase in PPC contributed nearly equally to the decline in DP over summer, and we estimated that, on average, PPC reduced digestibility of protein by 46%. However, the impact of PPC on digestibility was highly dependent
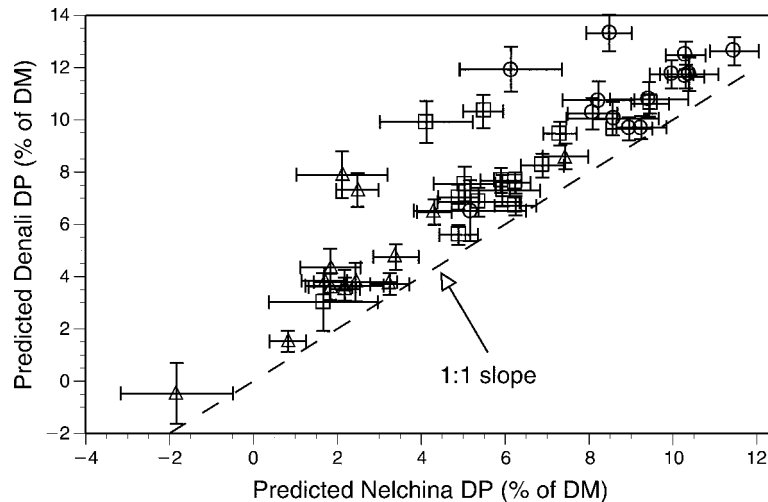
FIG. 3. The relationship between predicted digestible protein (DP) as a percentage of dry matter in Denali browses and Nelchina browses based on ANCOVA linear models (see *Methods and materials*). Circles represent estimates for Julian date 170 (21 June), squares represent estimates for Julian date 200 (20 July), and triangles represent estimates for Julian date 230 (20 August). Error bars represent the predicted SE for each estimate.

upon N concentration in the leaves. In early summer, when N concentrations were highest and PPC levels were lowest, PPC reduced protein digestion by as little as 5%, whereas in late summer, PPC reduced protein digestion by as much as 240%, indicating that consumption of these browses resulted in a net loss of protein to moose.

We estimate that DP was, on average, 23% lower in Nelchina than in Denali browse (5.33% of DM vs. 6.89% DM in Nelchina and Denali browses, respectively; $F_{1,1} = 19.0$, $P < 0.0001$; Table 1). Average DP was 26% lower ($P < 0.0001$) in browse leaves in 2004 than in leaves collected in either 2003 or 2001 (which were not significantly different in DP). Among species, we found that the average DP was highest in *S. alaxensis* (8.51% of DM), followed by *B. nana* (6.92% of DM). Average DP in the remaining three willow species was similar and significantly less than the DP of *S. alaxensis* and *B. nana*, ranging from 5.8% in *S. richardsonii* to 5.4% in *S. glauca* (Table 1).

Average DP was greater in Denali than in Nelchina browse in every year (Table 1). However, significant interactions of region × sample date occurred in four out of 12 individual species × year comparisons between regions (Appendix A: Table A1), which can potentially confound ANCOVA interpretations. Thus, to examine further how robust regional comparisons of DP were to interactions within the data, we compared digestible protein concentrations of each browse in each region and year on each sample date (i.e., at Julian dates 170, 200, and 230; Julian day 1 = 1 January), representing average early-, mid-, and late-summer nutrition) based on linear ANCOVA predictions. Predicted DP values for all species across all years were, on average, 1.5 times higher for Denali than for Nelchina, and in all but two cases, the SE of the estimates did not overlap the 1:1

regression line (Fig. 3). In two cases (*S. alaxensis* in 2004 and *B. nana* in 2003), the estimated DP concentrations in Denali leaves were up to 3.7 times higher than in respective samples of those plants consumed at the same time in Nelchina, and mean DP for each species was never predicted to be higher in Nelchina than in Denali.

Protein nutrition of moose

Our simulation model of moose protein balance on both the "Denali diet" and the "Nelchina diet" predicted nearly linear declines in net protein over summer, due to increased protein requirements for lactation in early summer and the decline in DP in all foods as summer progressed. Regardless of which simulated diet was consumed in which region, moose of the same reproductive status gained more lean body mass (or lost less lean body mass) over summer in Denali than in Nelchina each year (Table 2). In all but two cases ("Denali diet," one calf, 2004; "Denali diet," two calves, 2004), Denali moose of all reproductive states were predicted to gain at least some lean body mass over summer (Table 2), whereas in Nelchina only cows without calves consistently gained lean body mass.

In all simulated years, moose in both regions achieved consistently higher net protein intakes and greater protein reserves at the end of summer on the "Nelchina diet." On this diet, cows in Denali ended summer with ~16–27 kg more lean body mass (4–6% of body mass) and reached zero net protein intake 23 days later than cows in Nelchina, on average. Conversely, on the simulated "Denali diet," cows in Denali ended summer with ~7–23 kg more lean body mass and reached zero net protein intake 10 days later than cows in Nelchina. Finally, Denali moose consuming the simulated "Denali diet" ended summer with ~1–26 kg more lean body mass and reached zero net protein intake 8 days later

TABLE 2. Comparison of predicted change in lean body mass of cow moose (*Alces alces*) experiencing three reproductive states in the Denali and Nelchina study areas based on a simulation model of digestible protein intake over summer.

	Lean body mass gain (kg)†					
	No calves		One calf		Two calves	
Diet and year	Denali	Nelchina	Denali	Nelchina	Denali	Nelchina
Denali diet						
2001	23.7	9.9	11.6	−3.6	4.3	−12.2
2003	21.3	2.5	9.0	−11.8	1.5	−21.1
2004	4.6	−2.7	−9.4	−17.5	−18.6	−27.3
Nelchina diet						
2001	35.6	19.9	24.7	7.4	18.6	−0.2
2003	22.5	0.0	10.3	−14.5	3.0	−24.1
2004	24.6	3.3	12.6	−10.9	5.5	−20.2

Notes: Moose were simulated feeding on a mixed diet of five browse species, using seasonal trajectories of diet quality specific to each browse species, year, and region, as determined in this study. Simulations were run using two mixtures of browse species, based on field observations of moose diet composition in each study area (Denali diet and Nelchina diet).

† The predicted cumulative lean body mass gained over the simulation period, 20 June 20–1 September of each year.

than Nelchina moose consuming the "Nelchina diet" (Table 2, Appendix A: Table A3).

DISCUSSION

Temporal and regional patterns in browse nutrient status

Over the three years of this study, we found that the principal browses of moose in Denali National Park and Nelchina Basin in south-central Alaska varied significantly in protein precipitating capacity, nitrogen concentration, and digestible protein between species, years, regions, and sample dates throughout summer (Table 1). We found that PPC increased in browse leaves over summer by an average of 47%, whereas N concentrations decreased by an average of 36%. As a consequence, DP in these browses declined from an average of 9.8% in early summer to a low of ~3% by late summer, an average decline of ~70%. Browse digestible protein was consistently higher in Denali than in Nelchina for each species, year, and sample period (Table 1, Fig. 3). Furthermore, we found that tannins reduced browse protein availability by 46%, on average; hence they probably play a key role in the summer nitrogen balance of moose in these regions.

Although the nutritional phenologies of the browses were generally similar, with PPC increasing as N and DP declined roughly linearly over summer (Figs. 1 and 2, Appendix B: Fig. B1, Appendix C: Fig. C1, Appendix D: Fig. D1), differences in browse nutritional characteristics were confounded by interactions among regions, years, species, and sample dates (Table 1, Appendix A: Tables A1 and A2). These interactions highlight the complexity of plant chemical responses that browsing herbivores must contend with over the summer, among years, and among regions, and that probably impact the nutritional well-being of moose on these ranges. We suggest that the overarching nutrition-

al phenology pattern of the browses, and the more subtle patterns that induce region × year interactions, are due to a relatively modest suite of mechanisms. Low tannin concentrations in early spring are probably a response to high C demands of newly emerging leaves, low photosynthetic capacity, and perhaps competition within the phenylalanine pathway for protein synthesis rather than phenolic precursors to tannins (Jones and Hartley 1999). Differences in early-season N concentration of leaves may be a function of spring green-up date, which is in turn affected by winter snowpack as well as spring soil temperatures (Weih and Karlsson 2001, Welker et al. 2005, Aphalao et al. 2006). Indeed, the few large differences in N concentrations of leaves in early spring between years (e.g., *S. glauca* in Denali, Appendix C: Fig. C1, panel G) may reflect our sampling at different relative times after green-up.

Likewise, several mechanisms may be responsible for the variable rates of change of PPC and N concentration in leaves over summer. Differences in summer weather conditions that affect air and soil temperatures, soil moisture, and solar insolation (Weih and Karlsson 2001, Lenart et al. 2002, Aphalo et al. 2006) are likely to influence photosynthetic rates, growth rates, and C and N allocation within the plant. Carryover effects of the previous year's patterns of nutritional phenology are also possible. For example, nitrogen availability to plants has been shown to be influenced by the tannin concentrations of the previous year's plant litter (Joanisse et al. 2007). We cannot rule out the possible effects of species, year, or site-specific biotic interactions such as induced responses to herbivores (e.g., Markkola et al. 2004). Finally, phenological responses of N and PPC may partly depend on each other via dynamic physiological processes within the plant (Bryant et al. 1983; but see, e.g., Hamilton et al. 2001).

PLATE 1.   *Alces alces* (moose) twins. Photo credit: John Schoen.

*The impact of browse nitrogen dynamics
on moose nutritional status*

In spite of the complex nutritional responses of browses in this study, our data suggest a strong and consistent difference in browse PPC, N, and DP concentrations between Denali (where moose reproductive output was relatively good) and Nelchina (where reproductive output was poor). Our ANCOVA models predicted that, over summer and for each browse species, Denali forages provided between 11% and 37% more DP than the same forage species in Nelchina (Fig. 3). Because DP declines significantly over summer in virtually all browses, it was not surprising to find that net protein intake by moose reflected this phenological progression. Our simulations suggest that, regardless of which simulated diet was consumed, cow moose in Denali were predicted to be in positive protein balance longer, and to enter fall with greater lean body mass in every year (Table 2, Appendix A: Table A3). Furthermore, in all but two cases, Denali moose were predicted to gain at least some lean body mass over summer, whereas in Nelchina, only cows without calves consistently gained lean body mass over summer (Table 2). These results are consistent with the hypothesis that protein limits moose productivity in these two regions.

We recognize that these simulations may not accurately reflect the lean body mass dynamics of cows on these two summer ranges. Certainly, diet switching through summer may compensate for varying levels of DP in forages as summer progresses. Likewise, our simulations begin 1–2 weeks after green-up each year (D. E. Spalinger, *personal observation*), which might lead to an underestimation of lean body mass accumulations and of the dates at which moose reach zero net protein balance. We declined to attempt to optimize DP intake in our simulation models or to forward-project our simulations because of lack of information to justify it. Therefore, a more comprehensive analysis of protein balance of moose on these ranges must await better information on early-season nutrition and the fine-scale dynamics of diet selection over summer.

Nevertheless, we submit that the relative differences in protein dynamics of Denali and Nelchina moose are reasonable, and that Nelchina moose are likely to be N limited. In spite of the possible behavioral compensations that moose might attempt (for example, selecting from among the most nutritious plants at each site and at each time period), it is unlikely that net protein intake can increase substantially for Nelchina moose compared to Denali moose for at least three reasons. First, Denali browse of all species is higher in quality than Nelchina browse at virtually every time point over the three years of this study (Fig. 3). Hence, all else being equal, Denali animals should always be able to select a higher quality

diet than their Nelchina counterparts. Moreover, because diet quality generally has a multiplier effect on intake rates due to higher digestive and gastrointestinal passage rates (White 1983, Robbins 1993), Denali moose are likely to consume greater quantities of browse than Nelchina moose, making our comparative nutritional estimates conservative. Second, we sampled only in sites that were occupied by moose, and hence it is likely that we sampled plants of higher quality than average on each range. Therefore, the selective ability of moose is at least partially compensated for in our results. Third, the browses with the highest DP concentrations in both regions are those that are often most scarce or that pose other constraints on foraging or digestion. *Salix alaxensis*, the species with the highest average DP concentration, was relatively scarce at the locations where we found moose foraging. In Nelchina, *S. alaxensis* comprised only 14% of the plants sampled and 18% of plants that were browsed, whereas these percentages were slightly higher in Denali (23% of plants sampled, 37% of plants browsed). On the other hand, *Betula nana*, which generally was higher in DP than most other species, is very abundant in both regions. However, it is known to contain high levels of phenolic resins, which may influence digestion or be toxic to the animals (Graglia et al. 2001). Hence, it is unlikely that these forages will comprise substantially higher proportions of the diet than is presently assumed.

Although differences in nutritional characteristics among species and years are probably due to inherent genotypic characteristics of the species and to yearly fluctuations in the biotic or abiotic environment, the reasons for the stark differences in protein nutrition between regions is less clear. Because we are comparing two different regions, it is possible that climatic differences leading to a staggered green-up could consistently offset the start point of leaf phenological progression and thereby confound our season-long comparisons. However, we have observed that green-up in Denali and Nelchina generally occurs within a few days of each other (D. E. Spalinger, *personal observation*) and these observations are supported by seven years of remote-sensing data (Markon 2001). Hence, it is likely that comparisons of Denali and Nelchina nutritional quality are not an artifact of phenological differences between regions.

In a broader sense, the complexity and patterns of PPC and N concentrations over summer and between species, regions, and years are intriguing. Although the underlying geological or climatic differences between Denali and Nelchina may account for the differences that we observe between regions, we cannot rule out the possibility that biotic interactions such as persistent, long-term browsing by moose and caribou may influence these differences as well (e.g., Markkola et al. 2004). For example, our browse scarring data suggest that Nelchina plants were browsed 13% more than Denali plants, possibly supporting a role for herbivore-induced changes in plant chemistry. Nevertheless, in spite of the complexity in plant responses, we conclude that the nutritional landscape for Denali moose is superior to that of Nelchina moose, and suggest it is likely that Nelchina moose, particularly females supporting calves, often face severe shortages of N in summer. It is clear that factors such as predation influence large herbivores in the boreal environment (Gasaway et al. 1992, Testa 2004). However, our results suggest that greater emphasis should be placed on elucidating the relative or interactive role of diet quality, particularly summer nitrogen availability, in the population dynamics and conservation of northern ungulates.

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APPENDIX A

Comparison of summer leaf nutritional characteristics between Denali National Park and the Nelchina Basin, Alaska, and between years, based on ANCOVA. This appendix also contains a table comparing the date of zero net protein intake of cow moose in three reproductive states in Denali vs. Nelchina based on a simulation model of digestible protein intake over summer (*Ecological Archives* E090-092-A1).

APPENDIX B

Protein precipitation capacity of browses of Denali and Nelchina study areas for 2001, 2003, and 2004 (*Ecological Archives* E090-092-A2).

APPENDIX C

Nitrogen concentration of browses of Denali and Nelchina study areas for 2001, 2003, and 2004 (*Ecological Archives* E090-092-A3).

APPENDIX D

Digestible protein concentration of browses of Denali and Nelchina study areas for 2001, 2003, and 2004 (*Ecological Archives* E090-092-A4).