Nitrogen allocation to offspring and milk production in a capital breeder

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Abstract. Nitrogen (N) is a limiting nutrient for many herbivores, especially when plant availability and N content are low during the period of maternal investment, which is common for arctic ungulates. We used natural abundance of N isotopes to quantify allocation of maternal nitrogen to neonatal calves and milk in wild migratory caribou (*Rangifer tarandus*). We contrasted female–calf pairs from two herds in northern Quebec/Labrador, Canada: Rivière-George herd (RG; low population size with heavy calves) and the Rivière-aux-Feuilles herd (RAF; high population size and small calves). We assessed whether females of both herds relied on body protein or dietary N to produce the neonatal calf and milk at calving and weaning. Female caribou of both herds relied mostly on body N for fetal development. RAF females allocated less body N to calves than did RG females (92% vs. 95% of calf N), which was consistent with the production of calves that were 8% smaller in RAF than in RG. Allocation of body N to milk was also high for both herds, similar at calving for RAF and RG females (88% vs. 91% of milk N, respectively), but lower in RAF than RG females (95% vs. 99% of milk N) at weaning, which was consistent with a small but significantly greater reliance on dietary N supplies to support milk production at weaning. Female caribou used body protein stores to ensure a constant supply of N for fetal growth and milk production that minimized the effects of trophic mismatches on reproduction. The combination of migration and capital investment may therefore allow females to produce calves and attenuate the effects of both temporal and spatial mismatches between vegetation green-up and calf growth, which ultimately would reduce trophic feedbacks on population growth. Our data suggest that small changes in maternal allocation of proteins over the long period of gestation produce significant changes in calf mass as females respond to changes in resources that accompany changes in the size and distribution of the population.

Key words: capital breeders; income breeders; isotopes; maternal allocation; migratory caribou; nitrogen; northern Quebec and Labrador, Canada; Rangifer tarandus.

INTRODUCTION

Most herbivores partly rely on body stores of fat and protein to satisfy high demands for energy and nitrogen (N) during reproduction (Stearns 1992, Barboza and Parker 2008, Wilson and Festa-Bianchet 2009). Adult females use a continuum of allocation strategies from "capital breeders" that rely completely on body stores for reproduction to "income breeders" that rely on food consumed during reproduction (Meijer and Drent 1999, Stephens et al. 2009). In northern environments, large herbivores such as caribou (*Rangifer tarandus*), elk/red deer (*Cervus elaphus*), and moose (*Alces alces*) are known to be capital breeders (Durant et al. 2005, Gustine et al. 2010). Most fetal growth occurs in late winter when maternal body stores are at their yearly minimum (Schwartz and Hundertmark 1993) and the quality and abundance of forage is low (Weixelman et

al. 1998, Post et al. 2003). Several species of mammals and birds migrate to seasonal ranges where increases in plant quality and abundance complement the increasing demands for reproduction (Cotton 2003, Sinclair et al. 2010, Jonzén et al. 2011). Capital investment may attenuate both temporal and spatial variations in seasonal plant production, and allow females to successfully reproduce. However, a combined strategy of migration and capital investment may also reduce trophic feedback on population growth and predispose these populations to large demographic variations (Clutton-Brock and Coulson 2002, Sinclair et al. 2010).

Factors affecting female body condition, such as individual traits, habitat quality, population size, and weather (Marra et al. 2005, Bonenfant et al. 2009, Jonzén et al. 2011), may influence allocation of maternal resources to clutch production in birds (Devries et al. 2008), or fetal growth and milk production in mammals (Pekins et al. 1998, Chan-McLeod et al. 1999). In several northern ungulates, maternal fat stores are positively related to pregnancy rate, fetal growth rate, birth mass, and neonate survival: red deer (Albon et al. 1986);

TABLE 1. Body mass of adult female migratory caribou (*Rangifer tarandus*) and their calves at calving and weaning.

Season/age class	Year	RG herd		RAF herd	
		Mean	SE	Mean	SE
Calving					
Adult females	2007	80.4	1.8	73.0	1.8
	2008	83.1	1.5	79.4	1.5
	2009	81.2	1.8	75.4	1.6
Calves	2007	6.2	0.2	5.2	0.2
	2008	6.1	0.2	5.8	0.2
	2009	5.8	0.2	5.35	0.2
Weaning					
Adult females	2009	100.1	2.5	91.3	1.4
Calves	2009	48.2	1.4	39.3	1.8

Notes: Data were collected from 2007 to 2009 on female–calf pairs from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds, northern Quebec and Labrador, Canada. Sample sizes: 2007 (calving), $n = 20$ pairs per herd; 2008 and 2009 (calving and weaning), $n = 15$ pairs per herd.

muskoxen *Ovibos moschatus* (Adamczewski et al. 1992); caribou and reindeer (Allaye-Chan 1991); and moose (Keech et al. 2000). Although fat is the major energy store (Barboza et al. 2009, Parker et al. 2009), protein stores are used to meet high demands for N deposition in the fetus and in milk (Pekins et al. 1998, Chan-McLeod et al. 1999). According to recent studies, proteins, rather than fat stores, could be the primary constraint on reproduction for wild ungulates such as *Rangifer* (Barboza and Parker 2008) or *Odocoileus virginianus*, white-tailed deer (Simard 2010).

Nitrogen is a limiting nutrient for many herbivores, especially when animals consume senescent plants that are low in N (White 1993, Barboza et al. 2009). In late winter, while fat stores are decreasing and forage N content is very low (Parker et al. 2005, Van der Wal 2006), daily requirements of parturient ungulate females increase by 20–40% for energy (Chan-McLeod et al. 1994) and by 23–43% for nitrogen (Barboza and Parker 2008). In environments with either variable or low sources of dietary N, maternal body protein stores remain the primary and most stable source of N for fetal development (Cook et al. 2004, Barboza and Parker 2006). Inadequate supplies of dietary N can deplete body mass and protein stores in female ungulates and reduce allocation of N to the fetus (Barboza et al. 2009), reducing fetal growth (Rognmo et al. 1983, Sams et al. 1996) and newborn survival (Albon et al. 1987, Adams 2005). A female's ability to direct N from her own tissues or from her diet to fetal growth is highly dependent on the quality of resources available in late pregnancy, which may depend on factors such as weather and population density.

The isotopic ratio of N in metabolites ($^{15}N/^{14}N$ relative to atmospheric N; $\delta^{15}N$) has been used to investigate trophic interactions (Kelly 2000), diet com-position (Codron and Codron 2009, Arnould et al. 2011), and migration patterns (Schell et al. 1988). Comparisons between the isotopic ratios of N in tissues and diet can also be used to identify the source of N used by a female for egg production (Hobson 2006), fetal growth, or milk production (Parker 2003, Parker et al. 2005). Body protein stores are primarily derived from skeletal muscles and internal organs (Gerhart et al. 1996) that have a higher $\delta^{15}N$ than dietary protein (Kelly 2000, Caut et al. 2009). This is particularly true for female caribou during winter, when they subsist primarily on lichens, which contain less protein than is required for maintenance (Parker et al. 2005), and have very low $\delta^{15}N$ (Ben-David et al. 2001, Gustine et al. 2011). Comparisons between $\delta^{15}N$ of the diet and maternal tissues have been used to establish isotopic discrimination factors and to quantify fine-scale allocation of N in captive *Rangifer* (Barboza and Parker 2006, 2008) that allow us to apply this method to animals in the wild.

We used isotopic abundance of N ($\delta^{15}N$) to evaluate the source of N allocated by wild female migratory caribou to their offspring and to milk production. We contrasted female–calf pairs from two herds: the Rivière-George herd (RG) and the Rivière-aux-Feuilles herd (RAF) (Table 1; see Taillon et al. 2011). We knew from previous studies (Taillon et al. 2011, 2012*a*) that, at calving, RG calves were heavier than RAF calves, suggesting that the production of smaller calves may result from insufficient body capital in pregnancy. Therefore, we aimed to contrast the two herds by assessing whether females relied on body protein or dietary N to produce the calf and milk at calving. We expected all female caribou to rely mainly on body N for fetal development. However, we predicted that females constrained by environmental conditions would be more reliant on dietary income, such that maternal allocation of N to calves at birth would be lower in RAF than RG. Because females should prioritize fetal development, we expected milk production to rely on dietary N for both herds, especially for the RAF, such that maternal allocation of N to milk would be greater in RG than RAF. We also examined the relationships between maternal and calf body condition and maternal allocation of N. This study is the first to describe fine-scale maternal allocation of N in wild populations of large, migratory ungulates. By using natural abundance of ^{15}N in multiple tissues to evaluate body protein and dietary nitrogen, we aimed to understand the effects of the combination of the strategies of migration and of capital investment on these populations that vary widely in size and distribution.

METHODS

Study area

The Rivière-George (RG) and Rivière-aux-Feuilles (RAF) migratory caribou herds range over nearly one million square kilometers in the taiga and tundra

ecosystem of northern Quebec and Labrador, eastern Canada (Boulet et al. 2007). The two herds are genetically alike (Boulet et al. 2007), but have shown contrasting changes in demography and individual body condition over the last few decades (Couturier et al. 2010). The RG herd increased from fewer than 60 000 individuals in the 1950s (A. Rasiulis, M. Festa-Bianchet, and S. D. Côté, *unpublished data*) to more than 823 000 in 1993 (Couturier et al. 1996), and then declined to ~385 000 in 2001 (Couturier et al. 2004) and 74 000 in 2010 (Quebec Government aerial count, *unpublished data*). The RAF herd increased from 56 000 in 1975 (Le Hénaff 1976) to ~628 000 in 2001 (Couturier et al. 2004), and was estimated at 430 000 in 2011 (Quebec Government aerial count, *unpublished data*).

Range use

In early spring, females leave their southern wintering range (51–53° N) and migrate 250–650 km (J. Taillon, M. Festa-Bianchet, and S. D. Côté, *unpublished data*) to reach calving grounds that are typically used from late May to early July (Taillon et al. 2012b). Females of the RG herd calve on the high tundra plateaus on the east side of the Quebec–Labrador Peninsula (57° N, 65° W); females of the RAF herd calve in the center of the Ungava Peninsula (61° N, 74° W) (Taillon et al. 2012b). Calving is a period of high energy requirements for females and high vulnerability to predation for new-borns (Bergerud and Page 1987), such that the choice of calving grounds can affect the early survival and growth of calves.

Measurements and sample collection

At calving of 2007, 2008, and 2009 (5–14 June), we collected mother–calf pairs of both herds (sample size 20 pairs per herd in 2007; 15 pairs per herd in 2008 and 2009). We located caribou by helicopter and collected pairs over the entire calving grounds (calving ground size, mean ± SE: 6500 ± 745 km^2 for RG; 54 500 ± 2700 km^2 for RAF), spacing out collection sites by several kilometers (distance between sites, mean ± SE: 21 ± 3 km for RG; 83 ± 12 km for RAF). Calves were reliably matched to females because mothers isolated themselves at parturition. Only females with a newborn calf (<2 days old) were sampled. Newborns were unsteady and unable to run, their hoof pads were barely worn, and the umbilical cord was still attached (Couturier et al. 2009). In 2009, we also collected mother–calf pairs of both herds at weaning (23–30 October; sample size 15 pairs per herd) while the animals were migrating in broad corridors (RG = 55–56° N, 62–65° W; RAF = 54–58° N, 70–74° W). At weaning, mother–calf pairs were identified from behavioral observations on the ground and were collected over the entire range covered by animals fitted with radio-collars (average distance between collection sites: 64 ± 9 km for RG; 147 ± 27 km for RAF). Caribou were culled to assess their body condition and health as part of

recent and ongoing studies (Taillon et al. 2011, 2012a, Ducrocq et al. 2012). Animal manipulations followed guidelines of the Canadian Council on Animal Care as approved by the Laval University Animal Care and Use Committee (#2008015-3).

We measured the total body mass and the peroneus muscle mass of females and calves. Total body mass was obtained using a spring scale (accuracy for adult females ±0.25 kg; calves ±0.1 kg). The peroneus muscles (*peroneus tertius* with *extensor digitorum longus* and *extensor digit III*) provide a good estimation of protein mass in caribou (Crête and Huot 1993). The peroneus muscles were extracted from the right hind leg and were weighed with a Pesola scale (±0.5 g; wet mass) to estimate body protein reserves (Huot 1988, Chan-McLeod et al. 1995). The peroneus mass was corrected for the body mass, as both measurements are highly correlated (Taillon et al. 2011). We estimated the age of females by counting cementum layers in incisor teeth (Hamlin et al. 2000), and noted the sex of calves.

Samples collected at calving and weaning, definitions of variables, and years of collection are listed in Table 2. Muscle was sampled (1 × 3 cm) from the abdomen (e.g., skeletal muscle adjacent to the last rib) of both mother and calf, frozen at −20°C, and then air-dried at 50°C. Whole blood was collected from the jugular or the heart with a needle and syringe to prevent contamination from other body fluids, and was transferred to evacuated tubes without additive (Vacutainer Systems, Becton Dickinson, Franklin Lakes, New Jersey, USA). Serum and clot were separated by decanting whole blood for 24 h at 5°C. Blood serum was transferred to cryovials and stored at −20°C for analysis. Excess serum was decanted from clotted blood to retain a clot that was mostly red blood cells and fibrin. Drained clots were frozen and lyophilized for analysis. We collected 5–20 mL of fresh milk from the mammary glands by hand stripping. Milk samples were frozen at −20°C and lyophilized to determine dry matter content. Fresh feces (minimum of 30 pellets: around 50 g) were collected directly from the rectum, stored in paper bags, and air-dried at 50°C.

LABORATORY PROCESSING OF SAMPLES

Preparation of serum samples

Blood serum samples were fractioned into protein precipitates, urea, and amino acid samples (see Gustine et al. 2011). We used sodium tungstate and centrifugation to precipitate and remove serum proteins (Nolan and Leng 1972), and ion exchange columns to separate amino acids from blood serum. Serum proteins and serum amino acid samples were collected in plastic tubes (Falcon tubes; Becton Dickinson, Franklin Lakes, New Jersey, USA), stored at −20°C, and lyophilized for isotope ratio mass spectrometry.

Preparation of fecal samples

Dried fecal samples were first ground in a Wiley mill through a #20 (1.25-mm) screen, and then ~1 g of fecal

TABLE 2. Definition, source material, and period sampled for the different isotopic parameters collected from female–calf pairs of migratory caribou (*Rangifer tarandus*) from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds, northern Quebec and Labrador, Canada.

| | | | | Period | | |
| | | | | Calving | | Weaning |
Isotopic parameter	Definition	Collection years	Source	Females	Calves	Females
Fecal fibers $\delta^{15}N$	fecal fibers	2007–2009	fecal samples	X		X
Serum amino acids $\delta^{15}N$	free amino acids	2008, 2009	blood serum	X		
Blood clot $\delta^{15}N$	red blood cells	2008, 2009	blood clot	X		
Serum proteins $\delta^{15}N$	proteins	2008, 2009	blood serum	X		
Muscle $\delta^{15}N$	muscle	2007–2009	muscle	X	X	X
Milk $\delta^{15}N$	milk	2009	milk	X		X
Diet F $\delta^{15}N$	dietary N estimated from fecal fibers	2007–2009	derived	X		X
Diet AA $\delta^{15}N$	dietary N estimated from serum amino acids	2008, 2009	derived	X		
P^{dcalf}	percentage of calf muscle $\delta^{15}N$ derived from maternal muscle $\delta^{15}N$	2007–2009	derived		X	
P^{dmilk}	percentage of milk $\delta^{15}N$ derived from maternal muscle $\delta^{15}N$	2009	derived	X		X

material from each sample was boiled in distilled water to remove N from endogenous secretions (Gustine et al. 2011). Fecal samples were rinsed in sealed filter bags (ANKOM Technology, Macedon, New York, USA) that were previously washed with petroleum ether and air-dried to remove any N associated with residues on the polyester (see Gustine et al. 2011). Bags with fecal material were rinsed individually and dried overnight at 50°C to constant mass for measures of dry matter (DM) content. The fecal residues were reserved for subsequent analysis by mass spectrometry. Ash content was determined by combustion in a muffle furnace at 500°C to determine organic matter as the difference between dry matter and ash. Total N content was determined with an elemental analyzer (CNS2000, LECO, St. Joseph, Michigan, USA). Nitrogen content of feces was expressed as percentage of dry matter and organic matter.

Isotope ratio mass spectrometry

Muscle, blood (i.e., serum proteins, serum amino acids, and clot), milk, and feces were dried in tin cups at 65°C for mass spectrometry. We used isotope ratio mass spectrometry to measure enrichment of ^{15}N ($^{15}N/^{14}N$; $\delta^{15}N$ in ‰) in reference to atmospheric N (Gannes et al. 1997). Isotopic analyses were conducted by the Alaska Stable Isotope Facility (University of Alaska–Fairbanks (UAF), Fairbanks, Alaska, USA) and the Forest Soils Laboratory (UAF) using a continuous-flow isotope ratio mass spectrometer (model 2020, Europa Scientific, Crewe, Chestershire, UK). The accuracy of standard assays for peptone from meat (P7750, Sigma, Milwaukee, Wisconsin, USA) was within 0.39‰ for $\delta^{15}N$ (reference value 7.00‰). Isotope ratios of samples (R_{sam}) were expressed in relation to the standard of air ($R_{std} = 0.0036765$ $^{15}N/^{14}N$) as δ (parts per thousand, ‰)

(Wolfe 1992). We used single analyses for most measures of $\delta^{15}N$ because replicate analyses of 54 samples of feces and muscle provided a low CV of $4.0\% \pm 0.1\%$. Chemical assays for N content of feces and milk were performed in duplicate and repeated when CV exceeded 5%.

Calculations and statistics

Deriving diet $\delta^{15}N$.—We derived the $\delta_{15}N$ of the diet (diet $\delta^{15}N$) from fecal fibers $\delta^{15}N$ (at calving and weaning) and from serum amino acids $\delta^{15}N$ (only calving) to provide two estimates of maternal diet over a similar time frame. Fecal fibers reflected undigested N from the diet in the previous 50–71 h (Lechner et al. 2010), whereas serum amino acids reflected dietary N that had been absorbed from the diet over the last few days (Pearson et al. 2003). In both cases, we partly corrected for the presence of endogenous N that may increase ^{15}N by using a discrimination factor as follows.

First, plant fibers from composite fecal samples were used to estimate diet $\delta^{15}N$ (Barboza and Parker 2008, Gustine et al. 2011). Feces are typically enriched in ^{15}N compared to the diet because they contain protein-precipitating compounds, intestinal tissue, undigested plant fiber, or rumen biota (Van Soest 1994). Gustine et al. (2011) previously determined that fecal fibers $\delta^{15}N$ of captive *Rangifer* sp. fed on mixed and 100% lichen diets were enriched (Δ(fecal fibers)) by 3.34‰ ± 1.17‰ (mean ± SD; $n = 26$). Therefore, we used Δ(fecal fibers) and the fecal fibers $\delta^{15}N$ to estimate diet $\delta^{15}N$:

$$\text{Diet F } \delta^{15}N = \text{fecal fibers } \delta^{15}N - \Delta(\text{fecal fibers})$$

where Δ(fecal fibers) = 3.34‰ ± 1.17‰. We also estimated diet $\delta^{15}N$ using serum amino acids $\delta^{15}N$ based on a current validation study (P. Barboza, *unpublished data*), which determined that serum amino

acids $\delta^{15}N$ of captive *Rangifer* sp. was enriched [Δ(amino acids)] by 2.00‰ ± 0.58‰ (mean ± SD; $n =$ 12 animals for 34 determinations of Δ) compared to the diet. Therefore, we used Δ(amino acids) and serum amino acids $\delta^{15}N$ to estimate diet $\delta^{15}N$ as follows:

$$\text{Diet AA } \delta^{15}N$$
$$= \text{serum amino acids } \delta^{15}N - \Delta(\text{amino acids})$$

where Δ(amino acids) = 2.00‰ ± 0.58‰.

Percentages of maternal body protein (P^d).—Following Barboza and Parker (2008), we assumed that N in the muscle of newborn calves is derived (as indicated by superscript "d") from two pools that differ in $\delta^{15}N$: maternal body protein and maternal diet. We used the isotopic ratio of N in maternal muscle at calving to represent the $\delta^{15}N$ of maternal protein stores used to sustain fetal growth. We therefore calculated the percentage of calf muscle (P^{dcalf}) derived from maternal protein (see Barboza and Parker 2008) at calving as

$$P^{dcalf} = \left[\frac{C - D}{M + \Delta(\text{control}) - D} \right] \times 100$$

where C is calf muscle $\delta^{15}N$, M is maternal muscle $\delta^{15}N$, and D is maternal diet $\delta^{15}N$. P^{dcalf} indicates the percentage of calf muscle derived from maternal body protein against maternal dietary N (D) as the alternate source of N. We used the two estimates of diet $\delta^{15}N$ from fecal fibers (diet F $\delta^{15}N$) and serum amino acids (diet AA $\delta^{15}N$) to calculate P^{dcalf}. This model, however, assumes that there is no discrimination of dietary N as it passes from mother to calf. Following Barboza and Parker (2008), we used a measure of isotopic discrimination of dietary N (Δ(control)), calculated from penned female reindeer fed on control N-diet through winter (Barboza and Parker 2008). Control females were provided the same diet through winter and were used to measure isotopic discrimination (Δ(control)) as the enrichment factor of blood cells from the calf over its mother: Δ(control) = 1.08 ± 0.38, mean ± SD (Barboza and Parker 2008). In these calculations, we used muscle as the source of maternal N for the calf muscle and assumed a similar enrichment of calf over mother for blood cells in captive reindeer.

We used a similar calculation to estimate the percentage of maternal milk proteins derived (superscript d) from maternal tissues (P^{dmilk}) at calving:

$$P^{dmilk} = \left(\frac{\text{Milk} - D}{M - D} \right) \times 100$$

where Milk is maternal milk $\delta^{15}N$, M is maternal tissues $\delta^{15}N$, and D is maternal diet $\delta^{15}N$. We used two sources of maternal N (M): maternal muscle $\delta^{15}N$ and serum proteins $\delta^{15}N$. There were two estimates of diet $\delta^{15}N$ (D): from fecal fibers (diet F $\delta^{15}N$) and serum amino acids (diet AA $\delta^{15}N$). We also calculated the percentage of maternal milk proteins derived from maternal tissues (P^{dmilk}) at weaning using maternal muscle $\delta^{15}N$ as the source of maternal N and diet F $\delta^{15}N$ as the source of dietary N.

Statistical analysis

Dietary and maternal endpoints.—We tested for the effects of year, herd, season (only 2009), and second-degree interactions on fecal N content, fecal ash content, fecal fibers $\delta^{15}N$, and serum amino acids $\delta^{15}N$ using general linear models (GLM procedure, SAS Institute 9.2). For fecal fibers $\delta^{15}N$, we also tested for the relationship with fecal N content. We compared the $\delta^{15}N$ signature of the different maternal tissues and dietary estimates using t tests. We tested for the effects of year, herd, season (only 2009), and second-degree interactions on three maternal tissues: maternal muscle $\delta^{15}N$, maternal blood clot $\delta^{15}N$, and maternal serum protein $\delta^{15}N$ (GLM procedure, SAS Institute 9.2). We also tested for the relationship between maternal muscle $\delta^{15}N$, maternal blood clot $\delta^{15}N$, and maternal serum protein $\delta^{15}N$.

Maternal products.—We used general linear models to test the effects of year, herd, female muscle $\delta^{15}N$, female diet $\delta^{15}N$ and second-degree interactions with herd and year on calf muscle $\delta^{15}N$ and female milk $\delta^{15}N$ (GLM procedure, SAS Institute 9.2). We tested for the effects of year and herd on P^{dcalf} and the effect of herd and season on P^{dmilk}. Percentages (P^{dcalf} and P^{dmilk}) were transformed to the arcsine of the square root to meet assumptions of normality and homogeneity of variance. From the previously selected models, we then tested for the effect of female traits (body mass, peroneus muscle mass, and age) on calf muscle $\delta^{15}N$, female milk $\delta^{15}N$, P^{dcalf}, and P^{dmilk} (GLM procedure, SAS Institute 9.2). All female traits were normalized to the mean (mean = 0; SD = 1) to control for differences among years (Schielzeth 2010).

We ensured that all variables met assumptions of normality and homogeneity of variance. All data are presented as mean ± SE. A level of $\alpha = 0.05$ was used to determine significance.

Results

Fecal nitrogen and ash content

Fecal N content was similar between herds at calving (overall mean ± SE: 1.8% ± 0.02% dry matter for RG, 1.7% ± 0.02% for RAF; $F_{2,94} = 2.76$, $P = 0.10$; Appendix A). Fecal N was higher at weaning (3.0% ± 0.5% dry matter) than at calving (1.8% ± 0.02% dry matter; $F_{1,52} = 443.26$, $P < 0.0001$; Appendix A), but similar between herds ($F_{1,24} = 2.20$, $P = 0.15$). Ash content was higher for RG (0.17% ± 0.008%) than RAF (0.10% ± 0.003%) for all years ($F_{1,93} = 36.40$, $P < 0.0001$; Appendix A). Ash content did not affect the comparison of fecal N contents among herds and periods because those comparisons were similar when fecal N was expressed on the basis of dry matter or organic matter (dry matter minus ash; Appendix A).
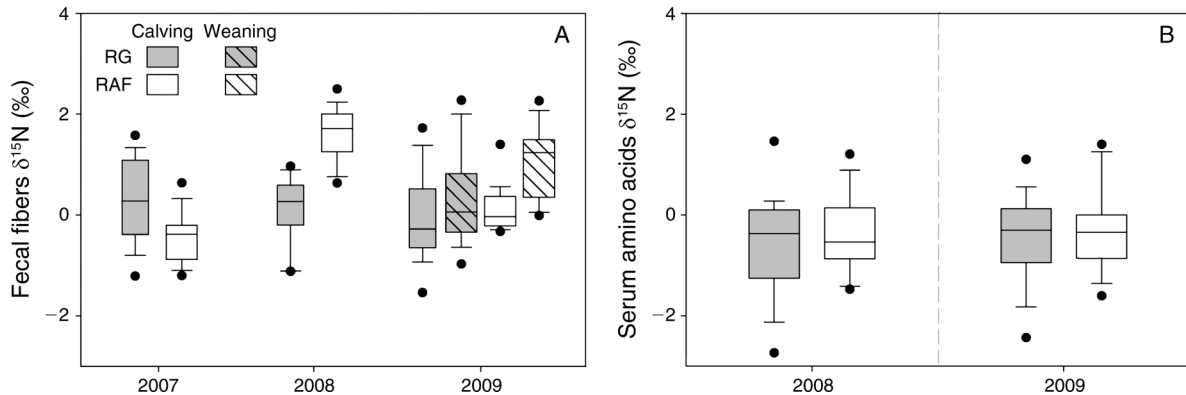
FIG. 1. Annual variations of isotopic indicators of dietary N in migratory caribou (*Rangifer tarandus*) from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds, northern Quebec and Labrador, Canada. (A) Data from fecal fiber $\delta^{15}N$ were collected on female–calf pairs from 2007 to 2009 during calving and during weaning in 2009. (B) Blood serum amino acids were sampled only in 2008 and 2009 during calving. Boxplots present the median (line), interquartile range (box), 90th percentiles (error bars), and 95th percentiles (dots).

Dietary endpoints

We used fecal fibers and serum amino acids to estimate dietary $\delta^{15}N$ of females at calving. Fecal fibers $\delta^{15}N$ was similar among years for RG females (overall: $0.16‰ \pm 0.11‰$, mean $\pm$ SE; $F_{1,47} = 0.92$, $P = 0.40$; Fig. 1A), but varied annually for RAF females (Fig. 1A). Overall, fecal fibers $\delta^{15}N$ was higher at weaning than at calving ($F_{1,52} = 9.07$, $P = 0.004$; Fig. 1A). There was a positive relationship between fecal fibers $\delta^{15}N$ and fecal N content for RG at both periods (for calving, slope = 2.05 ± 0.59; $t_{1,92} = 3.45$, $P = 0.0008$; for weaning, slope = 2.77 ± 1.22; $t_{1,22} = 2.27$, $P = 0.03$), but no relationship for RAF (for calving, $t_{1,92} = -0.32$, $P = 0.75$; for weaning, $t_{1,22} = 1.12$, $P = 0.28$).

Serum amino acids $\delta^{15}N$ was less variable than fecal fibers $\delta^{15}N$ (Fig. 1B) and similar between herds (overall $-0.49‰ \pm 0.17‰$ for RG, $-0.30‰ \pm 0.17‰$ for RAF; $F_{1,56} = 0.66$, $P = 0.42$) and years ($F_{1,56} = 0.23$, $P = 0.63$). The estimates of dietary $\delta^{15}N$ that were based on fecal

fibers (i.e., diet F $\delta^{15}N$, corrected for enrichment of fecal fibers) were lower (difference of $0.49‰ \pm 0.16‰$; $t_{1,56} = -3.08$, $P = 0.002$), but not significantly correlated with estimates of dietary $\delta^{15}N$ based on serum amino acids (i.e., diet AA $\delta^{15}N$, corrected for enrichment of serum amino acids) ($F_{1,56} = 1.46$, $P = 0.23$).

Maternal endpoints

We used muscle, blood clot and serum proteins to estimate maternal $\delta^{15}N$ at calving and weaning. All maternal tissues were enriched in ^{15}N and less variable than dietary endpoints from fecal fibers $\delta^{15}N$ or serum amino acids $\delta^{15}N$ (Figs. 1 and 2). At calving, maternal muscle $\delta^{15}N$ for RAF was similar among years ($F_{1,94} = 0.71$, $P = 0.49$) and not different to RG in 2007 and 2009 (all $P > 0.15$) (Fig. 2A). In 2008, RG maternal muscle $\delta^{15}N$ was higher than all other estimates ($F_{1,94} = 13.48$, $P < 0.0001$) (Fig. 2A). In 2009, maternal muscle $\delta^{15}N$ was similar at calving and weaning for RG females ($F_{1,27} = 0.18$, $P = 0.67$), but higher at weaning compared to
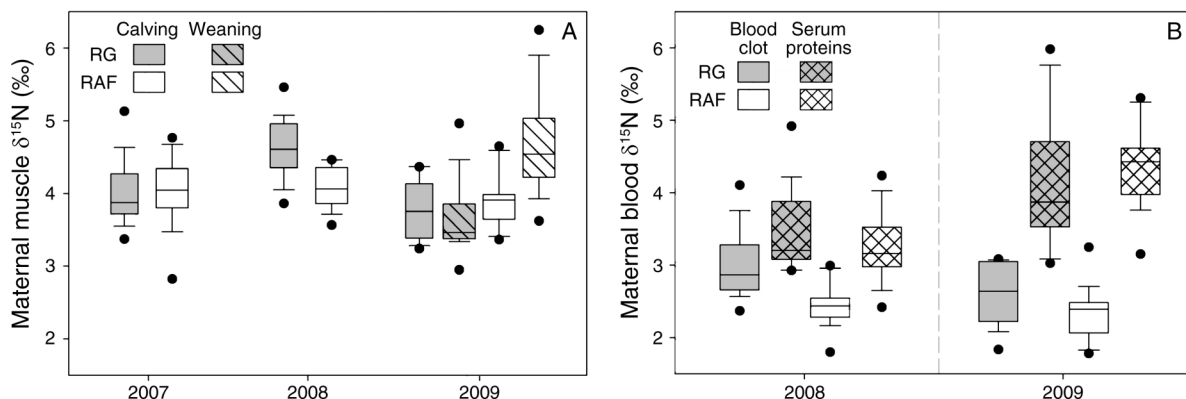

FIG. 2. Annual variations of isotopic indicators of maternal N from (A) muscle and (B) blood in migratory caribou from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds. Data were collected on female–calf pairs from 2007 to 2009. Blood samples (blood clots and serum protein) were taken in 2008 and 2009 only, during calving. Boxplots present the median (line), interquartile range (box), 90th percentiles (error bars), and 95th percentiles (dots).

calving for RAF females ($F_{1,23} = 11.78$, $P = 0.002$) (Fig. 2A).

At calving, maternal blood clot $\delta^{15}N$ was enriched for RG compared to RAF females ($F_{1,57} = 15.50$, $P = 0.0002$) (Fig. 2B). For both herds, maternal blood clot $\delta^{15}N$ was lower (for RG, $t_{1,28} = -15.61$, $P < 0.0001$; for RAF, $t_{1,29} = -22.09$, $P < 0.0001$; Fig. 2B), but positively correlated with maternal muscle $\delta^{15}N$ ($R^2 = 0.33$, $F_{1,57} = 16.42$, $P = 0.0002$). Maternal serum protein $\delta^{15}N$ was higher (for RG, $t_{1,29} = 7.77$, $P < 0.0001$; for RAF, $t_{1,29} = 10.09$, $P < 0.0001$; Fig. 2B), positively correlated with blood clot $\delta^{15}N$ (for herds grouped, $R^2 = 0.35$, $F_{1,57} = 52.53$, $P < 0.0001$), and sometimes higher than muscle $\delta^{15}N$ (Fig. 2), but was not correlated with maternal muscle $\delta^{15}N$ (for herds grouped, $F_{1,57} = 0.57$, $P = 0.45$).

Maternal products

Calf at birth.—Calf muscle $\delta^{15}N$ was higher than all maternal tissues $\delta^{15}N$ for both herds (all $P < 0.0001$) (Fig. 3A). The best regression model explained 41% of variance in calf muscle $\delta^{15}N$ at birth and included effects of year, herd, and maternal muscle $\delta^{15}N$ ($F_{4,93} = 15.87$, $P < 0.0001$). Calf muscle $\delta^{15}N$ varied annually, with highest estimates in 2008 ($F_{1,93} = 6.01$, $P = 0.0035$; Fig. 3A), and was higher over all periods for the RG (4.83‰ $\pm$ 0.07‰) than for the RAF herd (4.47‰ $\pm$ 0.07‰; $F_{1,93} = 6.01$, $P = 0.0035$; Fig. 3A). Similarly, for both herds, calf muscle $\delta^{15}N$ was positively related to maternal muscle $\delta^{15}N$ (overall slope = 0.50 ± 0.11; $F_{1,93} = 20.21$, $P < 0.0001$). There was no relationship between maternal diet F $\delta^{15}N$ and calf muscle $\delta^{15}N$ ($F_{1,92} = 1.94$, $P = 0.17$).

The percentage of calf muscle $\delta^{15}N$ (P^{dcalf}), derived from maternal muscle $\delta^{15}N$ vs. diet F $\delta^{15}N$, was higher for the RG (94.7% $\pm$ 0.8%) than for the RAF (91.9% $\pm$ 0.8%) ($F_{1,94} = 7.06$, $P = 0.009$; Fig. 3B) and did not differ among years ($F_{2,94} = 2.88$, $P = 0.06$; Fig. 3B). Changing the dietary endpoint (fecal fibers $\delta^{15}N$ vs. serum amino acids $\delta^{15}N$) in the calculation of P^{dcalf} did not affect the estimate for RAF ($t_{1,28} = 1.35$, $P = 0.09$) and only reduced the estimate by 0.9% $\pm$ 0.2% for RG ($t_{1,28} = 2.94$, $P = 0.003$). For both herds, maternal body mass, peroneus muscle mass, and age were not related to P^{dcalf} (for all tests, $P > 0.15$). Similarly, there was no relationship between calf birth mass or peroneus muscle mass and P^{dcalf} (for all tests, $P > 0.15$).

Milk.—There was no difference in milk protein content between herds at calving ($F_{1,26} = 0.49$, $P = 0.49$). At weaning, RG milk had slightly higher protein content compared to RAF milk (for RG, 12.06% $\pm$ 0.26%; for RAF, 11.22% $\pm$ 0.32% of wet mass; $F_{1,23} = 4.06$, $P = 0.056$), but protein content of milk was not correlated with milk $\delta^{15}N$ (for RG, $t_{1,14} = 0.01$, $P = 0.99$; for RAF, $t_{1,8} = 0.49$, $P = 0.54$). Milk $\delta^{15}N$ was lower than maternal muscle $\delta^{15}N$ for both herds at calving (all tests, $P < 0.0001$). At weaning, RG milk $\delta^{15}N$ was higher than maternal muscle $\delta^{15}N$ ($t_{1,14} = 2.33$, $P = 0.035$), whereas RAF milk $\delta^{15}N$ was similar to maternal muscle $\delta^{15}N$ ($t_{1,10} = 1.55$, $P = 0.15$; Figs. 2A and 4A).
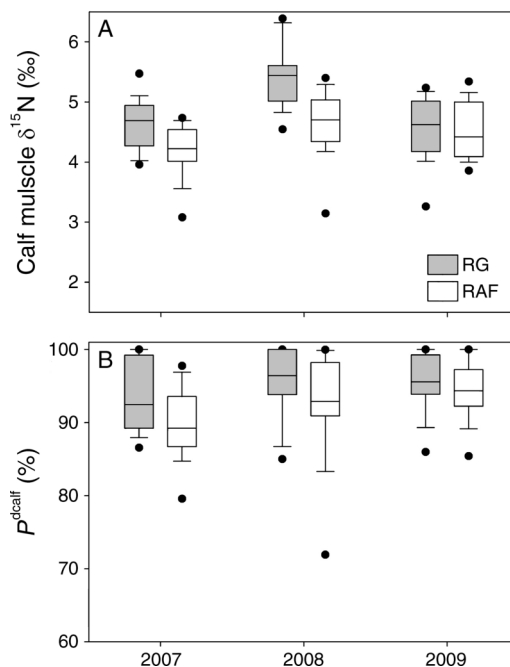

FIG. 3. Annual variations in (A) calf muscle $\delta^{15}N$ and (B) the percentage of calf muscle $\delta^{15}N$ (P^{dcalf}) derived from maternal muscle $\delta^{15}N$ at calving in migratory caribou from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds. Data were collected on female–calf pairs from 2007 to 2009. Boxplots present the median (line), interquartile range (box), 90th percentiles (error bars), and 95th percentiles (dots).

The best regression model explained 86% of the variance in milk $\delta^{15}N$ and included effects of season, herd, maternal muscle $\delta^{15}N$, and diet F $\delta^{15}N$ ($F_{4,49} = 72.49$, $P < 0.0001$). Milk $\delta^{15}N$ was higher at weaning (3.90‰ $\pm$ 0.07‰) than calving (3.24‰ $\pm$ 0.06‰) for both herds ($F_{1,49} = 44.38$, $P < 0.0001$; Fig. 4A), and was higher over all periods for the RG than for the RAF ($F_{1,49} = 10.15$, $P = 0.0025$; Fig. 4A). Milk $\delta^{15}N$ was positively related to muscle $\delta^{15}N$ (overall slope = 0.47 ± 0.09; $F_{1,49} = 27.96$, $P < 0.0001$) and diet F $\delta^{15}N$ (overall slope = 0.63 ± 0.06; $F_{1,49} = 110.00$, $P < 0.0001$) in a similar manner for both herds and seasons.

The percentage of milk $\delta^{15}N$ (P^{dmilk}), derived from maternal muscle $\delta^{15}N$ vs. diet F $\delta^{15}N$, was high for both herds and seasons (Fig. 4B) and was higher at weaning than at calving ($F_{1,49} = 40.46$, $P < 0.0001$; Fig. 4B). P^{dmilk} was similar for both herds at calving (for RG, 90.5% $\pm$ 1.5%; for RAF, 88.0% $\pm$ 1.2%) ($t_{1,26} = 1.61$, $P = 0.11$; Fig. 4B), but was higher for the RG than for the RAF at weaning (99.0% $\pm$ 0.7% vs. 95.3% $\pm$ 1.5%) ($t_{1,25} = 2.70$, $P = 0.009$; Fig. 4B). Changing the maternal endpoint (muscle $\delta^{15}N$ vs. serum proteins $\delta^{15}N$) decreased the estimates of P^{dmilk} at calving for both RG (difference of 6.7% $\pm$ 2.5%; $t_{1,13} = 2.66$, $P = 0.02$) and RAF (difference of 5.8% $\pm$ 1.6%; $t_{1,12} = 3.58$, $P = 0.004$), but did not affect the difference between seasons and herds. Changing the dietary endpoint (fecal fibers
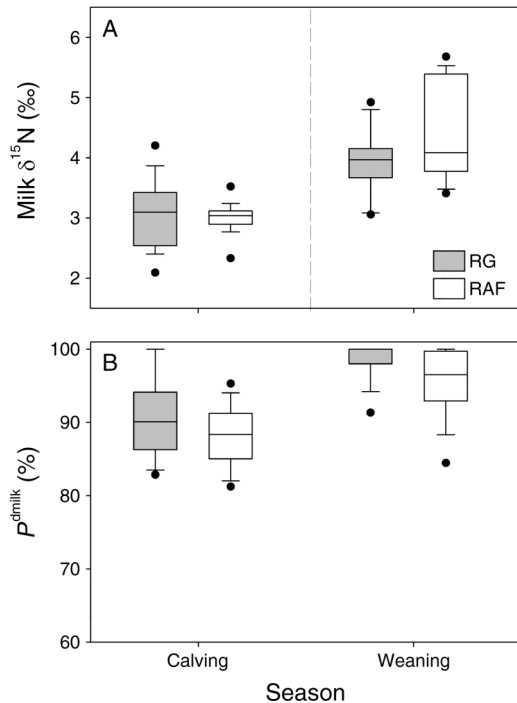
FIG. 4. Annual variations of (A) maternal milk $\delta^{15}N$ and (B) the percentage of milk $\delta^{15}N$ (P^{dmilk}) derived from maternal muscle $\delta^{15}N$ at calving and weaning for female migratory caribou from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds. Milk was only sampled in 2009. Boxplots present the median (line), interquartile range (box), 90th percentiles (error bars), and 95th percentiles (dots).

$\delta^{15}N$ vs. serum amino acids $\delta^{15}N$) slightly reduced the estimates of P^{dmilk} at calving for both RAF (difference of 1.9% $\pm$ 0.7%; $t_{1,12} = 2.74$, $P = 0.018$) and RG (difference of 1.9% $\pm$ 0.5%; $t_{1,13} = 3.48$, $P = 0.004$). For both herds at calving and weaning, maternal body mass, peroneus mass, and age were not related to P^{dmilk} (all tests, $P > 0.15$).

DISCUSSION

Female migratory caribou relied mostly on endogenous N for fetal development. Our data showed that RAF females allocated less body N to calves than did RG females (92% of calf N for RAF vs. 95% of calf N for RG), which was consistent with the production of smaller calves in RAF than in RG females (Table 1). Allocation of body N to milk was similar for both herds at calving (88% of milk N for RAF vs. 91% of milk N for RG), but lower in RAF than RG females, and was consistent with a small, but significantly greater, reliance on dietary N supplies to support milk production at weaning (95% of milk N for RAF vs. 99% of milk N for RG). Detailed knowledge of maternal allocation helps to explain the contrast in calf mass between the two herds, while the use of multiple tissues to evaluate body protein and dietary N provides guidance for future work using natural abundance of N isotopes in ungulates.

Diet quality and dietary endpoints

The range of estimates for dietary ^{15}N in our study is similar to measures of wild *Rangifer* in other locations based on direct measures of plants (−4.18‰ $\pm$ 0.92‰; Gustine 2011), ruminal contents (−1.28‰ to 0.20‰; McLeman 2006), and fecal fibers (−0.1‰ to 1.6‰ [Gustine et al. 2011]; −3.06‰ to 1.84‰ [Gustine 2011]). Low estimates of fecal fibers $\delta^{15}N$ at calving in both herds were consistent with a winter diet dominated by lichens (Ben-David et al. 2001, Finstad and Kielland 2011, Gustine et al. 2011). Higher fecal fibers $\delta^{15}N$, N, and ash content in feces at weaning compared to calving for both herds correspond to the switch to emergent vegetation available on the summer range, including sedges, forbs, and leaves of shrubs that are typically higher in N content and heavier in ^{15}N than are lichens (Drucker et al. 2010, Gustine 2011). Higher annual variation in ^{15}N of fecal fibers for RAF than for RG females may reflect greater variance in forage selection and movement over the summer range. Codron and Codron (2009) showed, in 16 African ungulate species, that fecal $\delta^{15}N$ tracks changes in plant ^{15}N, and fractionation primarily reflects variations in dietary protein. For both seasons, fecal N content and fecal fibers $\delta^{15}N$ were positively correlated for RG females only, which may be related to an earlier or greater access to emergent graminoids with high ^{15}N content than for the RAF females (Finstad and Kielland 2011). Interestingly, RG females have been found to arrive 2.6 $\pm$ 1.3 days earlier on calving grounds than RAF females (Taillon 2013). Differences in fecal ash content between RAF and RG females may also indicate differences in food selection as well as differences in both intake and digestibility of the diet (Barboza et al. 2009). This hypothesis awaits confirmation from further studies of diet composition in RAF and RG females, because ^{15}N of plants may vary regionally with hydrology, soil, and organic decomposition (Kelly 2000, Bump et al. 2009).

Serum amino acids $\delta^{15}N$ also indicated that RAF and RG females had a diet that was isotopically similar, but estimates were poorly correlated with dietary estimates from fecal fibers. This disparity probably reflects differences in the discrimination between diet and tissue as well as the turnover of metabolites in each isotopic pool (Caut et al. 2009, Martínez del Rio et al. 2009). The appearance of dietary N in the feces depends on food intake, microbial fermentation, and the low digestibility of certain proteins, whereas the appearance of dietary amino N in the serum depends on microbial N metabolism in the rumen, digestion, absorption, and net exchanges of amino acids between the gut and the liver (Lapierre et al. 2006, Reynolds 2006, Barboza et al. 2009). The estimation of dietary ^{15}N is therefore complex and best evaluated by more than one indicator. In this study, we used serum amino acids to confirm the estimates of dietary ^{15}N from fecal fibers to improve our estimates of N allocation from body against diet.

Maternal endpoints

All tissues $\delta^{15}N$ (muscle, blood clot, and serum proteins) were enriched compared to dietary $\delta^{15}N$ and similar to estimates from previous studies on wild *Rangifer*: values for muscle $\delta^{15}N$ of 2.5–3.0‰ (Barnett 1994) and 3.84–4.29‰ (McLeman 2006), and values for blood clot $\delta^{15}N$ of 1.0–3.5‰ (Ben-David et al. 2001, Finstad and Kielland 2011), 2.20‰ ± 1.56‰ (Gustine 2011), or 1.85–2.96‰ (Milakovic and Parker 2011).

At calving, muscle $\delta^{15}N$ was much higher and also less variable (range of 1‰) than fecal fibers $\delta^{15}N$ (range of 4‰). The contrast between maternal and dietary endpoints suggests that muscle $\delta^{15}N$ reflects the isotopic signature of previous late summer–fall vegetation (Barnett 1994, McLeman 2006, Finstad and Kielland 2011) and acts as a pool of N highly conserved for survival and allocation to gestation throughout winter (Barboza and Parker 2006, 2008). At weaning, RAF females presented higher muscle $\delta^{15}N$ than RG females, which is consistent with increased ^{15}N of the diet in summer (Gustine et al. 2011), but also suggests that body protein is conserved more in females at the higher population size. Accordingly, RAF females, currently at high population size, were 8 kg lighter and had lower kidney fat at weaning compared to RG females (Table 1; see Taillon et al. 2011).

Maternal allocation of nitrogen to offspring

The enrichment of calf muscle $\delta^{15}N$ over all maternal tissues $\delta^{15}N$ and the positive relationship with maternal muscle $\delta^{15}N$ indicated that maternal body protein was the primary source of N for fetal development. Similarly, Jenkins et al. (2001) found a general pattern of enrichment between red blood cells $\delta^{15}N$ of offspring and mothers during gestation and lactation for 11 species of mammals, including caribou, moose, and white-tailed deer. Our results on wild migratory caribou support recent studies on captive animals suggesting that *Rangifer* relies on body proteins for most fetal growth (Parker et al. 2005, Barboza and Parker 2006, 2008). Part of the variability in calf muscle $\delta^{15}N$, however, was not explained by maternal muscle $\delta^{15}N$ and could be explained by alternative pools of N, such as serum proteins or other pools of labile N in maternal organs during fetal development (Barboza and Parker 2006, Gustine et al. 2010; see Appendix B).

The percentage of calf muscle $\delta^{15}N$ derived from maternal stores (P^{dcalf}) was high for both herds and similar to estimates for captive *Rangifer* (Barboza and Parker 2008). Moreover, despite large variance in dietary endpoints, P^{dcalf} was not sensitive to changes in dietary $\delta^{15}N$. P^{dcalf} was ~3% higher for RG than for RAF females and, even if small, the difference was consistent with the production of lighter calves for RAF compared to RG females (difference of 0.7 kg or 8% of birth mass; Taillon et al. 2011, 2012*a*). These data indicate that small changes in allocation over a long gestation (210 to 230 days; Ropstad 2000) may subtend large changes in birth mass because 0.05% of maternal body protein is deposited in the fetus each day during the last 121 days of gestation (Barboza and Parker 2008). Lower estimates of P^{dcalf} probably reflect depleted maternal N stores in late gestation for the RAF females, which is consistent with lower body mass of RAF compared to RG females at calving (difference of 5.8 ± 0.4 kg; Taillon et al. 2011). The state of body stores determines the onset of reproduction in female caribou because body mass and body fat affect estrus and pregnancy (Cook et al. 2001, Langvatn et al. 2004). Accordingly, several studies on large ungulates showed that previous summer range condition and fall nutrition are the principal factors influencing female body condition during gestation (Couturier et al. 2009), conception rates (Simard et al. 2008), and birth masses (Kjellander et al. 2006). Subsequent development of the fetus is determined by the rate of maternal transfer of body stores, which is influenced by changes in the state of the store with season, food supply, and environmental conditions that often accompany shifts in size and distribution of the population (Barboza et al. 2009, Parker et al. 2009).

Maternal traits can also influence key life history variables of offspring, such as birth mass (Wilson and Festa-Bianchet 2009). Previous analyses on the same data set showed that calf birth mass was positively related to maternal body mass (Taillon et al. 2012*a*). For both herds, however, there was no relationship between calf $\delta^{15}N$, P^{dcalf}, and maternal traits (body mass, peroneus muscle mass, and age) or between maternal tissues $\delta^{15}N$ and calf birth mass (Finstad and Kielland 2011), probably because of the low variation in P^{dcalf} values and the large difference in total N of the mother and the calf (only 7% of maternal mass). A single measure of maternal mass and calf mass at parturition is probably insufficient to detect the effect of maternal traits on the rate of allocation over several weeks of gestation in capital breeders (Gustine et al. 2010) that can be revealed by isotopic differences between mother and offspring.

Maternal allocation of nitrogen to milk

For caribou females of both herds, variation in maternal milk $\delta^{15}N$ was partly explained by both maternal muscle $\delta^{15}N$ and diet F $\delta^{15}N$, indicating the contribution of both body and dietary proteins for milk production at calving and most likely also at weaning. Our results are consistent with the strong positive relationships found between milk $\delta^{15}N$ and maternal plasma $\delta^{15}N$, a proxy of dietary $\delta^{15}N$, in a variety of mammal species (Jenkins et al. 2001) and domestic cattle (Cheng et al. 2011). We found, moreover, that using serum proteins $\delta^{15}N$ as an alternative maternal endpoint explained more variation in milk $\delta^{15}N$ than did maternal muscle $\delta^{15}N$. This suggests that the production of milk involves alternative maternal-N pools along with the use of dietary N (Appendix B). Milk proteins are mainly

produced by the mammary gland from the amino acids present in the blood that are derived from both the turnover of maternal tissues and from the intestinal absorption of proteins from the diet and ruminal microbes (Lapierre et al. 2005).

As for P^{dcalf}, the percentage of milk $\delta^{15}N$ derived from maternal stores (P^{dmilk}) was high for both herds, indicating that maternal body protein was still the primary source of N allocated by females to milk production at calving. High P^{dmilk} at weaning suggests that maternal stores of N are crucial throughout lactation, even in late summer when females consume a diet higher in proteins (Crête et al. 1990, Gustine 2011). Our results conflict with previous work suggesting that lactating caribou allocate mostly surplus protein, not used for maintenance and storage, to milk production (Chan-McLeod et al. 1994, 1999), which agreed with allocation of only 19–34% of milk protein from body protein in domestic cattle (Wilson et al. 1988). We suggest that maternal stores ensure a supply of protein for milk production, whereas dietary income is more likely used to rebuild maternal stores. Our results are supported by previous studies showing poor relationships between milk protein content and diet quality prior to calving in reindeer (Rognmo et al. 1983) and in red deer during lactation (Landete-Castillejos et al. 2003). P^{dmilk} was also significantly higher for RG than RAF at weaning, when RAF females were 8 kg lighter than RG females (Taillon et al. 2011). Females at high population size (RAF) are likely to adopt a conservative strategy (e.g., "selfish cow strategy"; Russell et al. 1993) and to prioritize self-maintenance and future reproduction over current milk production (Chan-McLeod et al. 1999, Parker et al. 2009). Routing of N may change with the stage of lactation and the condition of the mother (Barboza and Parker 2006). Our observation of high $\delta^{15}N$ of milk proteins at weaning could reflect changes in the amount of N available to the mammary gland from sources of ingested N and circulating pools of protein and amino acids in blood when milk production declines while food intake increases to restore body mass in autumn. Furthermore, changes in milk output may be accompanied by shifts in isotopic discrimination between each source and the protein synthesized in milk. More information is required on the changes in maternal body tissue during lactation and the relative importance of diet and mobilized maternal tissues as sources of N for the synthesis of milk proteins. Future analyses of maternal blood components at weaning (i.e., serum amino acids, serum proteins, and blood clot) could help elucidate the role of alternative maternal-N pools in the production of milk.

From the income–capital breeding continuum, however, one might predict that use of body resources for reproduction would be greater in years or places where forage resources are not sufficient for reproductive needs (Meijer and Drent 1999, Stephens et al. 2009). On the contrary, females experiencing high population size and more nutritional constraints on reproduction (RAF) relied on dietary N to a greater extent than did females at low population size (RG) to produce smaller calves from similar reserves of maternal body mass. It is unlikely that the isotopic discrimination of N between the fetus and the sources of N in the mother and her diet are markedly different between these herds, because maternal diet and mass were similar between herds. Our data suggest that parturient caribou defend protein reserves that are probably used to sustain subsequent milk production and to survive to breed again. Small changes in allocation of dietary N to the fetus suggest that caribou use dietary N to complete fetal growth, that is, caribou rely on income to complete their capital investment in the fetus and to restore body capital at the end of the summer (Barboza and Parker 2008).

Similarly to maternal allocation of N to offspring, milk $\delta^{15}N$ and P^{dmilk} were not significantly related to maternal body mass, peroneus muscle mass, or age. Maternal traits, however, could be related to milk production and milk composition (Landete-Castillejos et al. 2003) that were slightly different between herds at weaning (J. Taillon et al., *unpublished data*).

Conclusion

Plant quality and biomass change dramatically from birth to weaning for most temperate large ungulates (Crête et al. 1990, Cook et al. 2004). Our data indicate that migratory caribou use plant production to restore their tissues, but rely on body protein to produce the calf and milk. Maternal investment of protein probably declines after peak milk output ~28 days after birth, as continued growth of the calf is mainly supported by the diet until weaning is completed in fall or early winter (Parker et al. 1990, Crête and Huot 1993). Heavy reliance on maternal body protein for offspring and milk production in large ungulates may reflect uncertainty in the timing of plant growth, peak biomass, and feeding opportunities because snowmelt, growing degree-days, and insect harassment vary widely over space and time (Russell et al. 1993, Fauchald et al. 2007). A reproductive strategy closer to the capital breeder continuum is therefore less affected by temporal mismatches between resource availability, here vegetation green-up, and birth date (Durant et al. 2005). We suggest that the combination of migration and capital investment may reduce the effect of trophic feedback on population growth and predispose these populations to large demographic variation.

The use of nitrogen isotopic ratios could help to refine our knowledge on maternal allocation in relation to reproductive strategies along the income–capital breeding continuum and under various environmental conditions in order to improve our understanding of trade-offs between reproduction and survival. Future research should contrast ungulate species along the income–capital breeding continuum to compare the use of

dietary and maternal pools in the production of offspring and milk.

Literature Cited

Adamczewski, J. Z., A. Gunn, B. Laarveld, and P. F. Flood. 1992. Seasonal changes in weight, condition and nutrition of free-ranging and captive female muskoxen. Rangifer 12:179–184.

Adams, L. G. 2005. Effects of maternal characteristics and climatic variation on birth masses of Alaskan caribou. Journal of Mammalogy 86:506–513.

Albon, S. D., T. H. Clutton-Brock, and F. E. Guinness. 1987. Early development and population dynamics in red deer. II. Density-independent effects and cohort variation. Journal of Animal Ecology 56:69–81.

Albon, S. D., B. Mitchell, B. J. Huby, and D. Brown. 1986. Fertility in female red deer (Cervus elaphus): the effects of body composition, age and reproductive status. Journal of Zoology 209:447–460.

Allaye-Chan, A. 1991. Physiological and ecological determinants of nutrient partitioning in caribou and reindeer. Dissertation. University of Alaska–Fairbanks, Fairbanks, Alaska, USA.

Arnould, J. P. Y., Y. Cherel, J. Gibbens, J. G. White, and C. L. Littnan. 2011. Stable isotopes reveal inter-annual and inter-individual variation in the diet of female Australian fur seals. Marine Ecology Progress Series 422:291–302.

Barboza, P. S., and K. L. Parker. 2006. Body protein stores and isotopic indicators of N balance in female reindeer (Rangifer tarandus) during winter. Physiological and Biochemical Zoology 79:628–644.

Barboza, P. S., and K. L. Parker. 2008. Allocating protein to reproduction in arctic reindeer and caribou. Physiological and Biochemical Zoology 81:835–855.

Barboza, P. S., K. L. Parker, and I. D. Hume. 2009. Integrative wildlife nutrition. Springer-Verlag, Berlin, Germany.

Barnett, B. A. 1994. Carbon and nitrogen isotope ratios of caribou tissues, vascular plants, and lichens from northern Alaska. Thesis. University of Alaska–Fairbanks, Fairbanks, Alaska, USA.

Ben-David, M., E. Shochat, and L. G. Adams. 2001. Utility of stable isotope analysis in studying foraging ecology of herbivores: examples from moose and caribou. Alces 37:421–434.

Bergerud, A. T., and R. E. Page. 1987. Displacement and dispersion of parturient caribou at calving as antipredator tactics. Canadian Journal of Zoology 65:1597–1606.

Bonenfant, C., et al. 2009. Empirical evidence of density-dependence in populations of large herbivores. Advances in Ecological Research 41:313–357.

Boulet, M., S. Couturier, S. D. Côté, R. D. Otto, and L. Bernatchez. 2007. Integrative use of spatial, genetic, and demographic analyses for investigating genetic connectivity between migratory, montane, and resident caribou herds. Molecular Ecology 16:4223–4240.

Bump, J. K., K. B. Tischler, A. J. Schrank, R. O. Peterson, and J. A. Vucetich. 2009. Large herbivores and aquatic–terrestrial links in southern boreal forests. Journal of Animal Ecology 78:338–345.

Caut, S., E. Angulo, and F. Courchamp. 2009. Variation in discrimination factors (Δ^{15}N and Δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46:443–453.

Chan-McLeod, A. C. A., R. G. White, and D. F. Holleman. 1994. Effects of protein and energy intake, body condition and season on nutrient partitioning and milk production in caribou and reindeer. Canadian Journal of Zoology 72:938–847.

Chan-McLeod, A. C. A., R. G. White, and D. E. Russell. 1995. Body mass and composition indices for female barren-ground caribou. Journal of Wildlife Management 59:278–291.

Chan-McLeod, A. C. A., R. G. White, and D. E. Russell. 1999. Comparative body composition strategies of breeding and non-breeding female caribou. Canadian Journal of Zoology 77:1901–1907.

Cheng, L., E. J. Kim, R. J. Merry, and R. J. Dewhurst. 2011. Nitrogen partitioning and isotopic fractionation in dairy cows consuming diets based on a range of contrasting forages. Journal of Dairy Science 94:2031–2041.

Clutton-Brock, T. H., and T. Coulson. 2002. Comparative ungulate dynamics: the devil is in the detail. Philosophical Transactions of the Royal Society B 357:1285–1298.

Codron, D., and J. Codron. 2009. Reliability of δ^{13}C and δ^{15}N in faeces for reconstructing savanna herbivore diet. Mammalian Biology 74:36–48.

Cook, R. C., J. G. Cook, and L. D. Mech. 2004. Nutritional condition of northern Yellowstone elk. Journal of Mammalogy 85:714–722.

Cook, R. C., D. L. Murray, J. G. Cook, P. Zager, and S. L. Monfort. 2001. Nutritional influences on breeding dynamics in elk. Canadian Journal of Zoology 79:845–853.

Cotton, P. A. 2003. Avian migration phenology and global climate change. Proceedings of the National Academy of Sciences USA 100:12219–12222.

Couturier, S., S. D. Côté, R. Otto, R. B. Weladji, and J. Huot. 2009. Variation in calf body mass in migratory caribou: the role of habitat, climate, and movements. Journal of Mammalogy 90:442–452.

Couturier, S., R. Courtois, H. Crépeau, L.-P. Rivest, and S. Luttich. 1996. Calving photocensus of the Rivière George Caribou Herd and comparison with an independent census. Rangifer 9:283–296.

Couturier, S., D. Jean, R. Otto, and S. Rivard. 2004. Demography of the migratory tundra caribou (Rangifer tarandus) of the Nord-du-Québec region and Labrador. Ministère des Ressources naturelles, de la Faune et des Parcs, Direction de l'aménagement de la faune du Nord-du-Québec et Direction de la recherche sur la faune. Quebec, Canada.

Couturier, S., R. D. Otto, S. D. Côté, G. Luther, and S. P. Mahoney. 2010. Body size variations in caribou ecotypes and

relationships with demography. Journal of Wildlife Management 74:395–404.

Crête, M., and J. Huot. 1993. Regulation of a large herd of migratory caribou: summer nutrition affects calf growth and body reserves of dams. Canadian Journal of Zoology 71: 2291–2296.

Crête, M., J. Huot, and L. Gauthier. 1990. Food selection during early lactation by caribou calving on the tundra in Quebec. Arctic 43:60–65.

Devries, J. H., R. W. Brook, D. W. Howerter, and M. G. Anderson. 2008. Effects of spring body condition and age on reproduction in Mallards (Anas platyrhynchos). Auk 125: 618–628.

Drucker, D. G., K. A. Hobson, J.-P. Ouellet, and R. Courtois. 2010. Influence of forage preferences and habitat use on ^{13}C and ^{15}N abundance in wild caribou (Rangifer tarandus caribou) and moose (Alces alces) from Canada. Isotopes in Environmental and Health Studies in Avian Biology 46:107–121.

Ducrocq, J., et al. 2012. Comparison of gross visual and microscopic assessment of four anatomic sites to monitor Besnoitia tarandi in barren-ground caribou (Rangifer tarandus). Journal of Wildlife Diseases 48:737–738.

Durant, J. M., D. O. Hjermann, T. Anker-Nilssen, G. Beaugrand, A. Mysterud, N. Pettorelli, and N. C. Stenseth. 2005. Timing and abundance as key mechanisms affecting trophic interactions in variable environments. Ecology Letters 8:952–958.

Fauchald, P., B.-J. Bardsen, K. Langeland, T. Tveraa, N. G. Yoccoz, and R. A. Ims. 2007. Escaping parasitism in the selfish herd: age, size and density-dependent warble fly infestation in reindeer. Oikos 116:491–499.

Finstad, G. L., and K. Kielland. 2011. Landscape variation in the diet and productivity of reindeer in Alaska based on stable isotope analyses. Arctic, Antarctic, and Alpine Research 43:543–554.

Gannes, L. Z., D. M. O'Brien, and C. M. Martínez. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology 78:1271–1276.

Gerhart, K. L., R. G. White, R. D. Cameron, and D. E. Russell. 1996. Estimating fat content of caribou from body condition scores. Journal of Wildlife Management 60:713–718.

Gustine, D. D. 2011. Protein status of muskoxen and caribou in late winter. Dissertation. University of Alaska–Fairbanks, Fairbanks, Alaska, USA.

Gustine, D. D., P. S. Barboza, L. G. Adams, R. G. Farnell, and K. L. Parker. 2011. An isotopic approach to measuring nitrogen balance in caribou. Journal of Wildlife Management 75:178–188.

Gustine, D. D., P. S. Barboza, and J. P. Lawler. 2010. Dynamics of body protein and the implications for reproduction in captive muskoxen (Ovibos moschatus) during winter. Physiological and Biochemical Zoology 83:687–697.

Hamlin, K. L., D. F. Pac, C. A. Sime, R. M. DeSimone, and G. L. Dusek. 2000. Evaluating the accuracy of ages obtained by two methods for Montana ungulates. Journal of Wildlife Management 64:441–449.

Hobson, K. A. 2006. Using stable isotopes to quantitatively track endogenous and exogenous nutrient allocations to eggs of birds that travel to breed. Ardea 94:359–369.

Huot, J. 1988. Review of methods for evaluating the physical condition of wild ungulates in northern environments. Nordicana. Series No. 50. Centre d'Études Nordiques, Université Laval, Quebec, Canada.

Jenkins, S. G., S. T. Partridge, T. R. Stephenson, S. D. Farley, and C. T. Robbins. 2001. Nitrogen and carbon isotope fractionation between mothers, neonates and nursing offspring. Oecologia 129:336–341.

Jonzén, N., E. Knudsen, R. D. Holt, and B.-E. Sæther. 2011. Uncertainty and predictability: the niches of migrants and nomads. Pages 91–109 in E. J. Milner-Gulland, J. M. Fryxell, and A. R. E. Sinclair, editors. Animal migration: a synthesis. Oxford University Press, Oxford, UK.

Keech, M. A., R. T. Bowyer, J. M. Ver Hoef, R. D. Boertje, B. W. Dale, and T. R. Stephenson. 2000. Life-history consequences of maternal condition in Alaskan moose. Journal of Wildlife Management 64:450–462.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Kjellander, P., J.-M. Gaillard, and A. J. M. Hewison. 2006. Density-dependent responses of fawn cohort body mass in two contrasting roe deer populations. Oecologia 146:521–530.

Landete-Castillejos, T., A. Garcia, J. A. Gomez, and L. Gallego. 2003. Subspecies and body size effect on lactation biology in red deer: comparison of Cervus elaphus hispanicus and C. e. scoticus. Physiological and Biochemical Zoology 76:594–602.

Langvatn, R., A. Mysterud, N. C. Stenseth, and N. G. Yoccoz. 2004. Timing and synchrony of ovulation in red deer constrained by short northern summers. American Naturalist 163:763–772.

Lapierre, H., R. Berthiaume, G. Raggio, M. C. Thivierge, L. Doepel, D. Pacheco, P. Dubreuil, and G. E. Lobley. 2005. The route of absorbed nitrogen into milk protein. Animal Science 80:11–22.

Lapierre, H., D. Pacheco, R. Berthiaume, D. R. Ouellet, and C. G. Schwab. 2006. What is the true supply of amino acids for a dairy cow? Journal of Dairy Science 89:E1–E14.

Lechner, I., P. S. Barboza, W. Collins, J. Fritz, D. Günther, B. Hattendorf, J. Hummel, K. H. Südekum, and M. Clauss. 2010. Differential passage of fluids and different-sized particles in fistulated oxen (Bos primigenius f. taurus), muskoxen (Ovibos moschatus), reindeer (Rangifer tarandus) and moose (Alces alces): Rumen particle size discrimination is independent from contents stratification. Comparative Biochemistry and Physiology A 155:211–222.

Le Hénaff, D. 1976. Inventaire aérien des terrains de vêlage du caribou dans la région nord et au nord du territoire de la municipalité de la Baie James (mai–juin 1975). Service de la recherche biologique, Ministère du Tourisme, de la Chasse et de la Pêche, Québec, Quebec, Canada.

Marra, P. P., C. M. Francis, R. S. Mulvihill, and F. R. Moore. 2005. The influence of climate on the timing and rate of spring bird migration. Oecologia 142:307–315.

Martínez del Rio, C., N. Wolf, S. A. Carleton, and L. Z. Gannes. 2009. Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84:91–111.

McLeman, C. I. A. 2006. Determining the relationships between forage use, climate and nutritional status of barren grounds caribou, Rangider tarandus groenlandicus, on Southampton Island, Nunavut, using stable isotopes analysis of δ^{13}N and δ^{15}N. Thesis. University of Waterloo, Waterloo, Ontario, Canada.

Meijer, T., and R. Drent. 1999. Re-examination of the capital and income dichotomy in breeding birds. Ibis 141:399–414.

Milakovic, B., and K. L. Parker. 2011. Using stable isotopes to define diets of wolves in northern British Columbia, Canada. Journal of Mammalogy 92:295–304.

Nolan, J. V., and R. A. Leng. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. British Journal of Nutrition 27:177–194.

Parker, K. L. 2003. Advances in the nutritional ecology of cervids at different scales. Ecoscience 10:395–411.

Parker, K. L., P. S. Barboza, and M. P. Gillingham. 2009. Nutrition integrates environmental responses of ungulates. Functional Ecology 23:57–69.

Parker, K. L., P. S. Barboza, and T. R. Stephenson. 2005. Protein conservation in female caribou (*Rangifer tarandus*): effects of decreasing diet quality during winter. Journal of Mammalogy 86:610–622.

Parker, K. L., R. G. White, M. P. Gillingham, and D. F. Holleman. 1990. Comparison of energy metabolism in relation to daily activity and milk consumption by caribou and muskox neonates. Canadian Journal of Zoology 68:106–114.

Pearson, S. F., D. J. Levey, C. H. Greenberg, and C. Martínez del Rio. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135:516–523.

Pekins, P. J., K. S. Smith, and W. W. Mautz. 1998. The energy cost of gestation in white-tailed deer. Canadian Journal of Zoology 76:1091–1097.

Post, E., P. S. Bøving, C. Pedersen, and M. A. MacArthur. 2003. Synchrony between caribou calving and plant phenology in depredated and non-depredated populations. Canadian Journal of Zoology 81:1709–1714.

Reynolds, C. K. 2006. Splanchic amino acid metabolism in ruminants. Pages 225–248 *in* K. Sejrsen, T. Hvelpund, and M. O. Nielsen, editors. Ruminant physiology: digestion, metabolism and impact of nutrition on gene expression, immunology and stress. Wageningen Academic Publishers, Wageningen, The Netherlands.

Rognmo, A., K. A. Markussen, E. Jacobsen, H. J. Grav, and A. S. Blix. 1983. Effects of improved nutrition in pregnant reindeer on milk quality, calf birth weight, growth, and mortality. Rangifer 3:10–18.

Ropstad, E. 2000. Reproduction in female reindeer. Animal Reproduction Science 60:561–570.

Russell, D. E., A. M. Martell, and W. A. C. Nixon. 1993. Range ecology of the Porcupine caribou herd in Canada. Rangifer Special Issue 8:1–168.

Sams, M. G., R. L. Lochmiller, C. W. J. Qualls, D. M. J. Leslie, and M. E. Payton. 1996. Physiological correlates of neonatal mortality in an overpopulated herd of white-tailed deer. Journal of Mammalogy 77:159–190.

Schell, D. M., S. M. Saupe, and N. Haubenstock. 1988. Natural isotope abundances in bowhead whale (*Balaena mysticetus*) baleen: markers of ageing and habitat usage. Pages 260–269 *in* P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, editors. Stable isotopes in ecological research. Springer-Verlag, New York, New York, USA.

Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. Methods in Ecology and Evolution 1:103–113.

Schwartz, C. C., and K. J. Hundertmark. 1993. Reproductive characteristics of Alaskan moose. Journal of Wildlife Management 57:454–468.

Simard, A. 2010. Dynamique de population d'un ongulé nordique à haute densité. Les déterminants environnementaux de la démographie et des composantes biodémographiques. Thèse de doctorat. Université Laval, Québec, Quebec, Canada.

Simard, M. A., S. D. Côté, R. B. Weladji, and J. Huot. 2008. Feedback effects of chronic browsing on life-history traits of a large herbivore. Journal of Animal Ecology 77:678–686.

Sinclair, A. R. E., K. Metzger, J. S. Brashares, A. Nkwabi, G. Sharam, and J. M. Fryxell. 2010. Trophic cascades in African savanna: Serengeti as a case study. Pages 255–274 *in* J. W. Terborgh and J. A. Estes, editors. Trophic cascades. Predators, prey, and the changing dynamics of nature. Island Press, Washington, D.C., USA.

Stearns, S. C. 1992. The evolution of life history histories. Oxford University Press, Oxford, UK.

Stephens, P. A., I. L. Boyd, J. M. McNamara, and A. I. Houston. 2009. Capital and income breeding: their meaning, measurement, and worth. Ecology 90:2057–2067.

Taillon, J., V. Brodeur, M. Festa-Bianchet, and S. D. Côté. 2011. Variation in body condition of migratory caribou at calving and weaning: Which measures should we use? Ecoscience 18:295–303.

Taillon, J., V. Brodeur, M. Festa-Bianchet, and S. D. Côté. 2012a. Is mother condition related to offspring condition in migratory caribou at calving and weaning? Canadian Journal of Zoology 90:393–402.

Taillon, J., M. Festa-Bianchet, and S. D. Côté. 2012b. Shifting targets in the tundra: protection of migratory caribou calving grounds must account for spatial changes over time. Biological Conservation 147:163–173.

Taillon, J. 2013. Condition physique, allocation maternelle et utilisation spatio-temporelle des aires de mise bas du caribou migrateur, *Rangifer tarandus*. Thèse de doctorat. Université Laval, Québec, Quebec, Canada.

Van der Wal, R. 2006. Do herbivores cause habitat degradation or vegetation state transition? Evidence from the tundra. Oikos 114:177–186.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Second edition. Cornell University Press, Ithaca, New York, USA.

Weixelman, D. A., R. T. Bowyer, and V. van Ballenberghe. 1998. Diet selection by Alaskan moose during winter: effects of fire and forest succession. Alces 34:213–238.

White, T. C. R. 1993. The inadequate environment: nitrogen and the abundance of animals. Springer, Berlin, Germany.

Wilson, A. J., and M. Festa-Bianchet. 2009. Maternal effects in wild ungulates. Pages 83–103 *in* D. Maestripieri and J. M. Mateo, editors. Maternal effects in mammals. University of Chicago Press, Chicago, Illinois, USA.

Wilson, G. F., D. D. S. Mackenzie, and I. M. Brookes. 1988. Importance of body tissues as sources of nutrients for milk synthesis in the cow, using ^{13}C as a marker. British Journal of Nutrition 60:605–617.

Wolfe, R. R. 1992. Radioactive and stable isotope tracers in biomedicine: principles and practice of kinetic analysis. Wiley-Liss, New York, New York, USA.

SUPPLEMENTAL MATERIAL

Appendix A

Annual values of N content in organic matter at calving in fecal samples and composition of fecal samples (fecal nitrogen and ash content) collected from adult female migratory caribou (*Rangifer tarandus*) from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds, northern Quebec and Labrador, Canada (*Ecological Archives* E094-165-A1).

Appendix B

Details on the enrichment of ^{15}N in maternal tissues, from blood clot to serum protein to muscle, of migratory caribou females (*Rangifer tarandus*) from the Rivière-George (RG) and Rivière-aux-Feuilles (RAF) herds, northern Quebec and Labrador, Canada (*Ecological Archives* E094-165-A2).