Sequential analysis of 15N in guard hair suggests late gestation is the most critical period for muskox calf recruitment

Schmidt, Niels Martin; Michelsen, Anders; Hansen, Lars Holst; Aggerbeck, Marie Ronne; Stelvig, Mikkel; Kutz, Susan; Mosbacher, Jesper Bruun

Published in:
Rapid Communications in Mass Spectrometry

DOI:
10.1002/rcm.9470

Publication date:
2023

Document version:
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC

Citation for published version (APA):
Sequential analysis of $\delta^{15}$N in guard hair suggests late gestation is the most critical period for muskox calf recruitment

Niels Martin Schmidt $^1$ | Anders Michelsen $^{2,3}$ | Lars Holst Hansen $^1$ | Marie Rønne Aggerbeck $^4$ | Mikkel Stelvig $^5$ | Susan Kutz $^6$ | Jesper Bruun Mosbacher $^7$

1Department of Ecoscience and Arctic Research Centre, Aarhus University, Roskilde, Denmark
2Department of Biology, University of Copenhagen, Copenhagen Ø, Denmark
3Center for Permafrost, University of Copenhagen, Copenhagen K, Denmark
4Department of Environmental Science, Aarhus University, Roskilde, Denmark
5Copenhagen Zoo, Frederiksberg, Denmark
6Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada
7Norwegian Polar Institute, Framsenteret, Tromsø, Norway

Correspondence
N. M. Schmidt, Department of Ecoscience and Arctic Research Centre, Aarhus University, Frederiksbergvej 399, DK-4000 Roskilde, Denmark.
Email: nms@ecos.au.dk

Funding information
Danmarks Grundforskningsfond, Grant/Award Number: CENPERM (DNRF 100);
Miljøstyrelsen: Greenland Ecosystem Monitoring Programme; Aarhus University;
Carlsberg Foundation, Grant/Award Number: CP17-0097; Brdr. Hartmann Foundation,
Grant/Award Number: A32156: 15. Juni Fonden; Danish National Research Foundation,
Grant/Award Number: DNRF 100; Danish Environmental Protection Agency

Rationale: Analysis of stable isotopes in tissue and excreta may provide information about animal diets and their nutritional state. As body condition may have a major influence on reproduction, linking stable isotope values to animal demographic rates may help unravel the drivers behind animal population dynamics.

Methods: We performed sequential analysis of $\delta^{15}$N values in guard hair from 21 muskoxen (Ovibos moschatus) from Zackenberg in high arctic Greenland. We were able to reconstruct the dietary history for the population over a 5-year period with contrasting environmental conditions. We examined the linkage between guard hair $\delta^{15}$N values in 12 three-month periods and muskox calf recruitment to detect critical periods for muskox reproduction. Finally, we conducted similar analyses of the correlation between environmental conditions (snow depth and air temperature) and calf recruitment.

Results: $\delta^{15}$N values exhibited a clear seasonal pattern with high levels in summer and low levels in winter. However, large inter-annual variation was found in winter values, suggesting varying levels of catabolism depending on snow conditions. In particular $\delta^{15}$N values during January–March were linked to muskox recruitment rates, with higher values coinciding with lower calf recruitment. $\delta^{15}$N values were a better predictor of muskox recruitment rates than environmental conditions.

Conclusions: Although environmental conditions may ultimately determine the dietary $\delta^{15}$N signal in muskox guard hairs, muskox calf recruitment was more strongly correlated with $\delta^{15}$N values than ambient snow and temperature. The period January–March, corresponding to late gestation, appears particularly critical for muskox reproduction.

1 | INTRODUCTION

The use of stable isotope analysis in animal ecology has long been recognized as a powerful tool to unravel animal diets from various tissues or excreta. In addition, stable isotope ratios may provide information about the nutritional state, and more specifically, the nitrogen (N) balance of individuals: dietary proteins are depleted in $^{15}$N compared to body proteins, and starving animals therefore show increased nitrogen isotope ratios ($\delta^{15}$N) as body stores are being catabolized. Starvation-induced increases in $\delta^{15}$N values have been...
reported for a variety of taxa, including mammals, birds, fish, and arthropods. In mammals, sequential stable isotope analysis of continuously growing hair allows for the creation of dietary chronologies over extended periods with high temporal resolutions, yielding detailed insights into seasonal and inter-annual fluctuations in diets and the periods of scarcity that may affect the fitness of individuals and ultimately the dynamics of populations. In ungulates, the nutritional state strongly influences individual fitness, and larger females generally live longer and have higher reproductive success. Ungulate nutritional state is therefore also tightly linked to vital population demographic rates.

High-latitude ungulates face extreme seasonality in forage accessibility and quality, with a short snow-free period with plenty of nutritious forage followed by an extended period of resource scarcity. In many northern ungulates the gestation period occurs over winter, and females must balance maintenance of their own body condition and reproduction. This is also the case for the muskox (Ovibos moschatus), the largest herbivore in the Arctic inhabiting some of the world’s most extreme environments, both in terms of seasonality and with respect to climatic conditions. As in other ungulates, muskox population dynamics is primarily governed by recruitment. It is therefore important to be able to determine which factors and periods are the most influential for muskox recruitment rates. In the present study we examine whether sequential analysis of \( \delta^{15}N \) in muskox hair can be used to map the starvation history and to pinpoint periods of the year that are particularly critical for successful reproduction. We have previously reconstructed a dietary chronology for muskoxen in high arctic Greenland covering approximately 2.5 years using sequential analysis of stable N isotope ratios in guard hairs. Here we extent this chronology to 5 years characterized by highly contrasting environmental conditions. Specifically, we map the intra- and inter-annual variation in muskox \( \delta^{15}N \) and ask which periods of the year are most closely linked to muskox calf recruitment rates, and thus most critical for muskox population dynamics in the region.

## EXPERIMENTAL

### Study area and sample collection

Data for this study were collected at Zackenberg in Northeast Greenland (74°28’N, 20°34’W). Zackenberg is located in the high arctic, with a mean annual air temperature of \(-9\,^\circ\text{C}\) and average annual precipitation of c. 260 mm, mainly falling as snow, and with considerable intra- and inter-annual variability. The Zackenberg area comprises wet, mesic, and dry tundra heaths, grasslands, and fens in the valley lowland, surrounded by abrasion plateaus and rocky outcrops at higher altitudes. The growing season typically extends from early June to early September, but onset may vary by several weeks from year to year according to the amount of snow precipitation. The muskox population is monitored within an approximately 47 km\(^2\) census area every summer to obtain data on muskox demographic parameters. In particular calf recruitment varies markedly across years. Muskoxen stay in the area around Zackenberg year-round.

In connection with a long-term GPS collaring project, we collected guard hair samples from 21 immobilized adult, female muskoxen (10 and 11 in 2013 and 2015, respectively). Following Mosbacher et al., guard hairs were cut from the rump region at the base of the skin using an electric hair clipper and placed in individual ziplock polyethylene bags until further processing in the laboratory (see below). Capture and handling of muskoxen was approved by the Government of Greenland (permit numbers G13–029 and G15–019), and all animals were immobilized with minimal pathophysiological responses.

### Chemical analyses of hair samples

Guard hairs were processed following Mosbacher et al. Briefly, qiviut was removed from the hair samples, then guard hairs were fixed in agarose gel, cut into 2 mm sections, and were washed in 96% ethanol. After being air-dried for at least 24 h, hair segments were then packed individually into tin caps each holding between 0.3 and 1.0 mg and analyzed using an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, Cheadle Hulme, Stockport, UK) coupled to a CN elemental analyzer (Eurovector, Milan, Italy) with continuous flow. The natural abundance of \( ^{15}N \) was expressed as \( \delta^{15}N \) (‰) = 1000 \( (R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}} \) where \( R = \text{mass 29/mass 28} \), and the standard had previously been calibrated against atmospheric \( N_2 \). By definition, atmospheric \( N_2 \delta^{15}N \) is 0‰. Samples were analyzed with reference gas calibrated against standards from International Atomic Energy Agency IAEA N1, N2 and US Geological Survey USGS 25, 26, and drift correlated using peach leaves (NIST 1547) from US National Institute of Standards and Technology (NIST) as an internal standard.

### Data analysis

All statistical analyses and graphics were conducted in R4.1.2. Before statistical analysis we filtered data for large outliers, potentially caused by contamination or analytical errors during sample preparation and stable isotope analysis. Of the 2205 segments analyzed, we omitted 68 presumed erroneous measurements/contamination (i.e., 3%). Remaining \( \delta^{15}N \) data were then standardized within individuals (mean = 0, standard deviation = 1). Each 2 mm hair segment corresponds to 9 days, and the dietary chronology was aligned with local time series of air temperature (°C) and snow depth (m) with known time stamp. Air temperature (°C) and mean snow depth (m) were recorded hourly from an automatic weather station located centrally in the Zackenberg valley. As the dietary chronology for muskox individuals covered up to a little more than 2.5 years, there was a small temporal overlap between the chronologies constructed based on the two capturing events.
We analyzed the correlation between hair $\delta^{15}$N signatures in all hair segments and calf recruitment and environmental conditions (air temperature and snow depths), using a random intercept linear mixed effect models with Satterthwaite correction in the “lme4” package. Muskox ID and Year were included as random effects, whereas air temperature and snow depth were fixed effects. As we expected responses to environmental conditions to be nonlinear, air temperature and snow depth were included as both linear and quadratic terms. The most parsimonious model was selected based on Akaike information criterion (AIC) using the “cAIC4” package.

To examine the potential link between hair $\delta^{15}$N signatures and demographic rates, we calculated the mean hair $\delta^{15}$N in 3-month sliding windows across all individuals as a measure of the population-level $\delta^{15}$N values and then correlated these with estimates of annual calf recruitment rates. We also examined whether any carryover effects could be detected by examining the correlation between previous year's hair $\delta^{15}$N signature and present year's calf recruitment rate. Calf recruitment rates (number of calves per 100 adult females) were estimated annually in July and August within the approximately 47 km$^2$ census area at Zackenberg as part of the ongoing monitoring there.

To evaluate whether environmental conditions were a better predictor of muskox demographics than hair $\delta^{15}$N, we also correlated the mean air temperature and snow depths against calf recruitment for all sliding windows.

3 | RESULTS

The sequential isotopic analysis of muskox guard hair allowed us to reconstruct the dietary history of muskoxen over a period of 5 years.

**FIGURE 1** The monthly standardized $\delta^{15}$N values across muskox individuals (upper panel), mean monthly snow depths (middle panel) and mean monthly air temperature (lower panel) at Zackenberg between 2010 and 2015. In the box plot, the horizontal line indicates the median, and the filled area corresponds to the first and third quartiles. Note that variation is attributable to both variations between individuals and within months [Color figure can be viewed at wileyonlinelibrary.com]
with a temporal resolution of approximately 9 days (Figure 1). The standardized \( \delta^{15}N \) mean monthly values varied from \(-1.00\%\) to \(0.64\%\) and exhibited clear seasonal as well as inter-annual fluctuations (Figure 1). Overall, the pattern in \( \delta^{15}N \) values followed that of the environmental conditions, in particular temperature but also snow depths. In addition to the random effects, the most parsimonious model included the linear terms for both air temperature and snow depth. Therefore, \( \delta^{15}N \) values in guard hairs were positively correlated with both air temperature (F1,217.6 = 76.719, P < 0.001) and snow depth (F1,1791.3 = 6.968, P < 0.008). The monthly fluctuations in the unstandardized \( \delta^{15}N \) values are given in Figure S1 (supporting information).

Examining the linkage between the various 3-month sliding window and muskox demographics revealed that the \( \delta^{15}N \) values in December–February and January–March were significantly negatively correlated with calf recruitment (\( R^2_{adj} = 0.70, P = 0.049 \) and \( R^2_{adj} = 0.83, P = 0.020 \), respectively; Figure 2). Remaining periods were not significantly correlated with calf recruitment (\( R^2_{adj} < 0.41, P > 0.147 \); Figure 2). Similarly, we found no indications of a carryover effect as previous year’s \( \delta^{15}N \) values and present year’s calf recruitment rates were not correlated in any of the time windows (\( R^2_{adj} < 0.05, P > 0.349 \)).

The correlations between environmental conditions (air temperature and snow depth) and muskox calf recruitment were not significant in any of the 3-month periods (\( R^2_{adj} < 0.53, P > 0.099 \) for air temperature [January–March]; \( R^2_{adj} < 0.63, P > 0.067 \) for snow depth [March–May; Figure S2 [supporting information]]). It is noteworthy that snow depth and calf recruitment were negatively correlated in three time windows, though only marginally significant so (January–March: \( P = 0.083 \), February–April: \( P = 0.068 \), March–May: \( P = 0.067 \)).

4 | DISCUSSION

Understanding the drivers of animal demographics and population size is essential in wildlife management and conservation. For ungulates, including muskoxen, calf recruitment is one of the main determinants of population dynamics. By constructing a 5-year dietary chronology for the muskox population at Zackenberg in high arctic Greenland, we show that the nutritional condition in winter, and in particular January through March, appears particularly critical for calf recruitment and thus for the dynamics of the muskox population.

FIGURE 2 Correlations between the mean standardized \( \delta^{15}N \) values in muskox guard hairs and annual calf recruitment rate (number of calves per 100 adult females) in the various 3-month sliding windows during the years 2010–2015. Lines are the linear trends, with full lines showing significant trends (\( P < 0.05 \)) and broken lines showing non-significant trends. Shaded areas are the 95% confidence intervals.
The dietary signal in muskox guard hairs followed the seasonal changes in environmental conditions, and in particular air temperature closely. $\delta^{15}N$ values in muskox hair are generally higher in summer compared to winter, reflecting the intake of $^{15}N$-rich nutritious graminoid forage in summer season and $^{15}N$-depleted low-quality forage in the snow-covered period. However, as snow depths increase, so do the $\delta^{15}N$ values in guard hairs. This suggests that with increasing snow, muskoxen to a greater extent must rely on catabolism of body stores. Muskoxen are well adapted to such seasonal variability in access to forage and build up large body stores during summer and autumn for somatic maintenance and reproduction. However, winter severity (here in terms of snow depths) differs between years, and so does access to forage in winter and the concomitant degree to which muskoxen draw upon stored resources. Muskoxen usually give birth from late March to mid-May, which also seems to be the case at Zackenberg. The strong correlation between $\delta^{15}N$ values in January-March and calf recruitment within the same year therefore suggests two things: (a) that the period prior to birth (late gestation) is the most critical period for muskox reproduction and (b) that starvation and concomitant catabolism of stored resources in late gestation results in lower recruitment of calves the following summer. We acknowledge that we only have a limited number of years in our study and that our measure of calf recruitment constitutes the end point of a series of demographic steps from conception to calf weaning. Nonetheless, previous research lends support to our findings: at Zackenberg most adult muskoxen appear to be in good conditions at the time of the rut and become pregnant irrespective of the environmental conditions. However, as winter severity increases, and with that the likelihood of starvation, the rate of fetal resorption and/or abortion goes up. Moreover, in the cases where females do give birth, poor maternal condition may also result in lower calf survival as lactation is energetically very costly. That maternal condition over winter appears to drive calf recruitment has also been observed in both other muskox populations and reindeer (Rangifer tarandus) populations. Specifically for muskoxen, the late gestation period seems to be key for successful reproduction. As many other ungulates, muskoxen thus appear to be maximizing survival and future reproduction rather than current reproduction. Non-pregnant females may reduce metabolic costs in winter further by lowering their body temperatures, thereby saving substantial amounts of stored resources. This not only increases their chances of survival, but it also makes them energetically better prepared for the next breeding season. However, we did not detect any signals of carryover effects in our analyses.

We have previously documented the linkage between muskox calf recruitment and snow conditions, and in the present study we also found marginally significant relationships between snow depth and muskox calf recruitment. However, the signal obtained from the analyses of hair appeared to be a better predictor of calf recruitment than environmental conditions, as we recently also showed for mineral levels in muskox qiviut. The muskox population at Zackenberg roams within the same area year-round. Differences in access to forage induced by the amount of snow, rather than changes in movement patterns per se, are therefore most likely causing the inter-annual variations in $\delta^{15}N$ values in muskox hair. Therefore, although environmental conditions are the ultimate cause of the varying $\delta^{15}N$ values observed in muskox guard hairs, the $\delta^{15}N$ values measured directly in hair with high time resolution seem to reflect the energetic condition of the animals better.

5 CONCLUSIONS

Using sequential stable isotope analysis of muskox guard hair, we reconstructed the dietary history of the muskox population at Zackenberg in high arctic Greenland and have shown that starvation periods have negative consequences for muskox calf recruitment. In snow-rich years, starvation and concomitant catabolism of reserves seem to prevail (Figure 3), and late gestation emerged as particularly

![Diagram: Conceptual overview of the impacts of winter snow conditions on muskox catabolism of reserves, calf recruitment, and guard hair $\delta^{15}N$ values. Full lines indicate causal relationships, whereas the dotted line indicates non-causal association. “+” indicates “increasing,” whereas “−” indicates “decreasing.”]
critical for successful reproduction in muskoxen. Climate has shaped muskox population dynamics and distribution for millennia, and muskoxen are well adapted to life under arctic conditions. However, the projected increases in arctic precipitation may severely challenge muskox reproduction and ultimately population viability.

ACKNOWLEDGMENTS

The authors thank Greenland Ecosystem Monitoring Programme for access to and logistics at Zackenberg. The authors are indebted to the Carlsberg Foundation (CF-17-0097), Brdr. Hartmann Foundation (A32156), 15. Juni Fonden, the Danish National Research Foundation for supporting CENPERM (DNRF 100), and the Danish Environmental Protection Agency for their financial support. Without their help, this project would not have been carried through. Finally, special thanks to Rune B. Mosbacher for the idea about how to fasten guard hair in gel to allow for the sequential cutting.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Niels Martin Schmidt https://orcid.org/0000-0002-4166-6218

REFERENCES

26. Mosbacher JB, Kristensen DK, Michelsen A, Stelvig M, Schmidt NM. Quantifying muskox plant biomass removal and spatial relocation of


**Supporting Information**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Schmidt NM, Michelsen A, Hansen LH, et al. Sequential analysis of δ¹⁵N in guard hair suggests late gestation is the most critical period for muskox calf recruitment. *Rapid Commun Mass Spectrom.* 2023;37(6): e9470. doi: 10.1002/rcm.9470