

## **Limited dietary overlap amongst resident Arctic herbivores in winter – complementary insights from complementary methods**

Niels M. Schmidt<sup>1\*</sup>, Jesper B. Mosbacher<sup>1</sup>, Eero J. Vesterinen<sup>2,3</sup>, Tomas Roslin<sup>2,4</sup>, Anders Michelsen<sup>5,6</sup>

<sup>1</sup> Arctic Research Centre, Department of Bioscience, Aarhus University, Frederiksborgvej 399, 4000 Roskilde, Denmark. <sup>2</sup> Department of Agricultural Sciences, Latokartanonkaari 5, FI-00014 University of Helsinki, Finland. <sup>3</sup> Biodiversity Unit, Vesilinnantie 5, FI-20014 University of Turku, Finland. <sup>4</sup> Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, 750 07 Uppsala, Sweden. <sup>5</sup> Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen, Denmark. <sup>6</sup> Center for Permafrost (CENPERM), University of Copenhagen, Øster Voldgade 10, 1350 Copenhagen K, Denmark

\* Corresponding author: E-mail: [nms@bios.au.dk](mailto:nms@bios.au.dk); Phone: +45 87 15 86 83

### **Authors contribution statement**

NMS, TR and AM conceived the ideas and designed methodologies; NMS and JBM collected samples. NMS, EJV and AM conducted the analyses; NMS and TR led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## **Abstract**

Snow may prevent Arctic herbivores from accessing their forage in winter, forcing them to aggregate in the few patches with limited snow. In High Arctic Greenland, Arctic hare and rock ptarmigan often forage in muskox feeding craters. We therefore hypothesized that due to limited availability of forage, the dietary niches of these resident herbivores overlap considerably, and that the overlap increases as winter progresses. To test this, we analyzed fecal samples collected in early and late winter. We used molecular analysis to identify the plant taxa consumed, and stable isotope ratios of carbon and nitrogen to quantify the dietary niche breadth and dietary overlap. The plant taxa found indicated only limited dietary differentiation between the herbivores. As expected, the dietary niches exhibited a strong contraction from early to late winter, especially for rock ptarmigan. This may indicate increasing reliance on particular plant resources as winter progresses. In early winter, the diet of rock ptarmigan overlapped moderately with that of muskox and Arctic hare. Contrary to our expectations, no inter-specific dietary niche overlap was observed in late winter. This overall pattern was specifically revealed by combined analysis of molecular data and stable isotope contents. Hence, despite foraging in the same areas and generally feeding on the same plant taxa, the quantitative dietary overlap between the three herbivores was limited. This may be attributable to species-specific consumption rates of plant taxa. Yet, Arctic hare and rock ptarmigan may benefit from muskox opening up the snow pack, thereby allowing them to access the plants.

**Keywords:** Competition, Greenland, Metabarcoding, Stable isotopes, Tundra

## Introduction

The resident herbivores on the Arctic tundra face harsh climatic conditions and limited forage availability in winter, and severe snow conditions may strongly limit their availability to forage and thus to gain sufficient energy to maintain their energy balance (Callaghan et al. 2011; Mosbacher et al. 2016b). How different herbivore species solve these challenges will have a critical impact on how they survive the winter, and how current changes in winter conditions (Bokhorst et al. 2016) may affect processes at the level of populations, behavior and interspecific competition, as all dictated by resource limitations.

Arctic herbivores cope with the challenging winter situation in rather different manners. Lemmings (*Lemmus* sp. and *Dicrostonyx* sp.), and other small mammals, live relatively protected in the sub-nivean space, with relatively easy access to food under the snow (Klein and Bay 1991). In fact, winter may be regarded as the prime time for lemmings (Schmidt et al. 2008). In contrast to this, surface-active resident herbivores remain exposed to the winter conditions, and consist of only a handful of species. Specifically, in High Arctic Greenland the surface-active herbivore community in winter comprises muskox (*Ovibos moschatus*, Zimmermann, 1780), Arctic hare (*Lepus arcticus*, Ross 1819) and rock ptarmigan (*Lagopus mutus*, Montin 1781) (Berg et al. 2008). As other cold-climate ungulates during winter, muskox rely heavily on fat reserves built up over summer and autumn (Adamczewski et al. 1998). During this time, forage is plentiful, particularly in the productive meadow areas (Kristensen et al. 2011; Mosbacher et al. 2016a). In contrast, the Arctic hare and the rock ptarmigan accumulate only small fat reserves during summer, and therefore need to forage regularly during winter to maintain the energy balance (Thomas 1987). Both species may dig small, temporary shelters in the snow, which offers some protection from the ambient conditions (Thomas 1987; Gray 1993).

Muskoxen, Arctic hares and rock ptarmigan are generally not regarded as competitors for food in winter due to expected differences in space use (Klein and Bay 1991, 1994; Schaefer et al. 1996). However, in High Arctic Greenland the three species are frequently observed foraging in the same areas during winter. There, the aggregation of foraging activities is mainly initiated by muskox cratering behavior. By digging through the snow pack, the muskox increases the access to the otherwise hidden vegetation for themselves, but ultimately also for other herbivore species. A similar phenomenon has been observed in Svalbard, where reindeer cratering opens up foraging areas for rock ptarmigan (Pedersen et al. 2006). The overlap in foraging area is likely to be particularly pronounced in periods with extensive snow cover (such as in late winter; Pedersen et al. 2016), when the availability of suitable foraging areas, and thus access to food, is particularly limited. This use of shared foraging sites could be hypothesized to result in a strong overlap in resource use.

Yet, the resident herbivores of the High Arctic also differ in several respects. Not only do they differ substantially in size and anatomy, conferring different modes of accessing and shredding plant material. Being phylogenetically distant (a bird, an ungulate and a lagomorph), they also rely on widely different modes of food digestion. The muskox is a ruminant, the Arctic hare is a hind-gut fermenter, and the ptarmigan uses enlarged ceca as fermentation chambers. This may cause them to focus on different nutritional resources, as being differentially amenable to digestion by these different strategies (Smith et al. 2000). Thus, the extent to which shared space use of these Arctic species also results in shared resource use is yet to be established.

Herbivore diet can be mapped out by several techniques. Most analyses of Arctic herbivore diet to date have relied on conventional techniques, scoring diet from plant remains in gut contents or feces (e.g. Thing et al. 1987; Klein and Bay 1991, 1994). Such techniques risk providing information of relatively low resolution, since a significant proportion of plant

material cannot be identified or can be assigned only to rough taxonomic groups (Norbury 1988). As an alternative, several studies have applied stable isotope ratios to infer diets (Nielsen et al. 2017). Here, the stable isotope ratio of a consumer is assumed to reflect that of its diet, with different primary producers often having distinct stable isotope ratios (Fogel and Cifuentes 1993). During assimilation and metabolism the lighter stable isotope is preferentially excreted, resulting in consumers being isotopically enriched relative to their prey. This phenomenon is commonly represented as a trophic discrimination factor, and has recently yielded a plethora of isotope-based studies quantifying animal resource use, primarily using carbon and nitrogen stable isotopes (Boecklen et al. 2011). Such analyses work well when putative dietary resources can be well established a priori, the isotope ratios of different primary producers are clearly differentiated and the trophic discrimination factor can be accounted for. However, they are less suited for offering species-level resolution (see Traugott et al. 2013; Phillips et al. 2014; Roslin and Majaneva 2016). As another alternative, recent molecular approaches rely on the locus-specific amplification of DNA fragments from the diet, and matching the resulting amplicons with a reference library (Soininen et al. 2009; Roslin and Majaneva 2016). The advantage of molecular approaches is their high taxonomic resolution. The precision of identifications improve with well-populated reference libraries. Here, the Arctic is in a good position, with extensive libraries of plants (data from Willerslev et al. 2014; Soininen et al. 2015). For Northeast Greenland, a complete reference library of all vascular plants was generated by Wirta et al. (2014, 2016). As disadvantages, molecular approaches tend to give qualitative lists of taxa consumed by each animal rather than estimates of quantities consumed – an aspect currently debated (Deagle et al. 2013; Clare 2014). Given the advantages and disadvantages of each individual technique, a powerful approach to unravel detailed trophic interactions is then to combine and compare approaches in the same study (Hardy et al. 2010).

To map out the winter-time diet compositions of the resident, surface-active herbivores of High Arctic Greenland, we adopt this joint approach. We combine molecular and stable isotope analyses of fecal samples to quantify differences in niche breadth and dietary overlap between species and seasons. In doing so, we focus on two contrasting periods of winter: early winter with fragmented, shallow snow-cover, and late winter with extensive, deep snow-cover. We hypothesize that due to the anatomical differences, the three herbivore species may consume different parts of the plants, but that due to the limited availability of plants (both in terms of biomass and species) in the snow-free areas, their diets may overlap considerably when foraging in the same areas. As winter progresses and access to resources declines, we expect the dietary niche breadth to decline and the dietary overlap between herbivore species to increase.

## **Materials and methods**

### *Study area*

The study was conducted at Zackenberg in Northeast Greenland (74°30'N; 21°00'W). Here, the climate is High Arctic with a mean annual temperature of -9°C, a mean summer (June through August) temperature of about +5°C and a mean winter (December through February) temperature of about -18°C. Annual precipitation is about 260 mm, mainly falling as snow (Hansen et al. 2008; Pedersen et al. 2016). Winter thaw events are rare in the area (Pedersen et al. 2015). The vegetation in the valley is dominated by various tundra heath types, intertwined by smaller, wind-exposed and sparsely vegetated areas (Elberling et al. 2008).

### *Collection of fecal material*

We collected fecal material from Arctic hare, muskox, and rock ptarmigan primarily within muskox feeding craters but also on smaller, windblown ridges, where the sparse vegetation was accessible for all three species. All samples were collected within approximately 6-7 km from the Zackenberg research station. Co-feeding was verified at all collection sites by the presence of fresh fecal droppings from all three species. Fecal samples were collected from individual piles (muskox and rock ptarmigan) or within areas separated by minimum 25 meters (Arctic hare). Fecal material from the three species is easily distinguishable based on shape, size and color. Only fresh feces were collected, based on coloration and position of fecal material on the snow surface. Fecal samples were placed in separate paper bags and oven-dried at 60°C until complete dryness. We collected 20 samples from each species in each of the two seasons, early winter (October 2013) and late winter (April 2012). Snow depth in the central valley was approximately 15 cm in October 2013 and approximately 100 cm in April 2012, respectively (Pedersen et al. 2016). Prior to analyses, fecal samples from the three herbivores were ground-milled into a homogenous powder.

### *Genetic analysis*

We used the same fecal material prepared for the analysis of stable isotopes (below) to conduct molecular analyses, thus allowing us to detect the plant taxa consumed by the three herbivores in the two seasons by both methods.

DNA extraction – For DNA extraction from fecal material, we subsampled 31.1 mg ( $\pm$  SD 3.8 mg) for DNA extraction. DNA was extracted using the NucleoSpin® Tissue Kit (product nr 740952, Macherey-Nagel), according to the instructions for standard protocol (User manual, version June 2014 / Rev. 14) with the following modifications: step 3) after incubation, an extra centrifugation for 5 min at 11 000 g to clear supernatant; step 5) centrifuge time was increased to 4 min to assure more complete flow-through; and step 7)

pure DNA was eluted into 200 µL Buffer BE. Four microliters of each extract were used for each PCR.

PCR and library construction – DNA libraries were prepared using the protocol from Vesterinen et al. (2016), with minor modifications. In short, we used two widely applied gene regions in our study: Firstly, chloroplast *trnL* (UAA) intron and 2) internal transcribed spacer 2 (*ITS2*). Firstly, *trnL* region was amplified using two forward primers (c\_A49325: 5'-CGAAATCGGTAGACGCTACG-3'; and g\_A49425: 5'-GGGCAATCCTGAGCCAA-3') and one reverse primer (h\_B49466 5'-CCATTGAGTCTCTGCACCTATC-3') producing two different products per sample (Taberlet et al. 2007). Secondly, *ITS2* region was similarly amplified using two forward (gITS7 5'-GTGARTCATCGARTCTTTG-3'; fITS7 5'-GTGARTCATCGAATCTTTG-3'; Ihrmark et al. 2012) and one reverse primers (ITS4 5'-TCCTCCGCTTATTGATATGC-3'; White et al. 1990). All these PCR products were then subduced to another PCR round to attach Illumina-specific adapters and to introduce sample-specific barcodes. We prepared two separate pools to account for PCR and sequencing artefacts. Sequencing was performed on Illumina MiSeq platform (Illumina Inc., San Diego, California, USA) in two separate runs by the Turku Centre for Biotechnology, Turku, Finland. Further details of the laboratory work are available in Appendix S1(Text S1.1).

Sequencing output analysis and OTU identification – The sequencing runs produced altogether 8 572 802 and 13 755 492 forward and reverse raw reads. The reads were then uploaded to CSC servers (IT Center for Science, [www.csc.fi](http://www.csc.fi)) for trimming and further analysis. Trimming and quality control of the sequences were carried out according to Vesterinen et al. (2016) with minor modifications for Illumina platform. We used USEARCH algorithms for trimming and clustering and python package cutadapt for primer cutting (Edgar 2010; Martin 2011). All commands and a more detailed description of the bioinformatics applied is offered in Appendix S1 (Text S1.2).

Altogether, 2 997 047 reads were retrieved for subsequent analysis for the current study. We tested the reliability and repeatability of the sequence data by applying t-test for read numbers for each data type (technical replicate A vs. B per each species; early winter vs. late winter per species). Then, OTUs were identified to biological taxa using BLAST (Altschul et al. 1990) at the CSC servers. We used several databases: a) local database ‘chgh’ consisting of chloroplast *trnL* sequences (data from Willerslev et al. 2014; Soininen et al. 2015) local database ‘ZACK’ consisting of plant *ITS2* sequences (Wirta et al. 2014, 2016) and c) current (August 2016) GenBank ‘nt’ database. Most of the OTUs (308/511) were identified with a 100% match to a single species. Overall, 99 % of trimmed and merged reads offered a match of at least 98% to species (or some times higher taxa) – hereafter referred to as plant taxa – and were retained. For subsequent analysis we combined the complementary information from different primers and data sets, that is, we considered a dietary plant taxon to be present in a sample, if it was found by any data set. Sequence data including OTU reads are available in the Dryad Digital Repository: <http://datadryad.org/resource/doi:XXXXXXXXXX>

### *Stable isotope analysis*

For the stable isotope analyses, subsamples were weighed (~3.5 mg fecal material) and packed into tin capsules. Subsamples were then analyzed for elemental concentrations and  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$  ratios. All analyses were conducted using an Isoprime isotope ratio mass spectrometer (Isoprime Ltd, Cheadle Hulme, Stockport, UK) coupled to a CN elemental analyser (Eurovector, Milan, Italy) with continuous flow. Natural abundances of isotopes are expressed in the  $\delta$  notation relative to international standards (Vienna Pee Dee Belemnite for C and atmospheric  $\text{N}_2$  for N):  $\delta X_{\text{sample}} (\text{‰}) = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1]$ , where R is the molar ratio of heavy X / light X. All samples were analysed with reference gas calibrated against international standards IAEA C5, CH6, CH7, N1, N2 and USGS 25, 26, 32, and drift corrected using peach leaves (NIST) as internal standard. The standard deviation of isotope

measurements of the standards was  $\pm 0.2\%$  for  $\delta^{15}\text{N}$  and  $\pm 0.1\%$  for  $\delta^{13}\text{C}$  (Clemmensen et al. 2006).

#### *Dietary niche breadth and dietary overlap*

From the molecular analyses, we first obtained a list of plant taxa found (presence/absence) in each of the fecal samples from the three herbivores in the two seasons. Based on the presence/absence of plant taxa we calculated the frequency of occurrence for each plant taxa for each herbivore species in each season. We then calculated the dietary niche breadth using Levin's measure ( $B$ ) (Krebs 1998):

$$B = \frac{1}{\sum P_j^2}$$

where  $P_j$  is the overall proportion (based on presence/absence in individual scats) of plant taxa  $j$  in the fecal samples of one of the three herbivore species in one of the two seasons. Levin's measure ranges from 1 (only one plant species consumed) to the maximum plant taxa consumed (i.e. all plant taxa consumed in equal amounts).

We did not include any analysis of the number of reads, as these may not reflect the importance of the various plant taxa in the herbivore diets (Pompanon et al. 2012). Moreover, the large anatomical differences between the three herbivore species may further exacerbate differences. Different plant groups may also amplify differently, introducing further bias in the absolute read counts.

From the data on stable isotope ratios, we calculated the dietary niche breadth for each species in early and late winter using the SIBER package by quantifying the area of the standard ellipses, which include the central 40% of the observations (Jackson et al. 2011) and

thus represents the dietary core. While the convex hull encapsulates all data points, and thus highly influenced by outliers (Layman et al. 2007), the area of standard ellipses yields more robust measures of diet niche (Syväranta et al. 2013). Hence, we use this area to describe the dietary niche of the three herbivores examined. As stable isotope ratios may altered while passing through the digestive tract (Sponheimer et al. 2003), we corrected the stable isotope ratios in feces for trophic enrichment prior to the statistical analyses (Table 1). We used published data on trophic discrimination factors from goats for the muskox ( $\delta^{13}\text{C}$ :  $-1.00\pm 1.00$ ;  $\delta^{15}\text{N}$ :  $1.70\pm 1.00$  (Codron et al. 2012)) and from rabbits for the Arctic hare ( $\delta^{13}\text{C}$ :  $-0.30\pm 0.10$  (Sponheimer et al. 2003);  $\delta^{15}\text{N}$ :  $2.00\pm 0.50$  (Boag et al. 1997)). For rock ptarmigan we used the general avian trophic discrimination factor ( $\delta^{13}\text{C}$ :  $1.15\pm 0.18$ ;  $\delta^{15}\text{N}$ :  $2.91\pm 0.16$ ) published by Caut et al. (2009). However, as these trophic discrimination factors are not specific to the species or tissues examined here, they may not adequately describe the trophic enrichment. We therefore also estimate the dietary overlap using uncorrected stable isotope ratios and compare the estimated overlaps with and without trophic discrimination factors applied. We used linear regression to compare the two measures of dietary width (i.e. area of the standard ellipse and Levin's  $B$ ).

To quantify the dietary overlap between the herbivore species using the molecular data, we calculated Schoener's overlap index (Hurlbert 1978),  $P$ :

$$P_{jk} = \left[ \sum_{i=1}^n (\text{minimum } P_{ij}, P_{ik}) \right] 100$$

where  $P_{i,j}$  and  $P_{i,k}$  are the proportions (here based on presence/absence) of plant taxa  $i$  in the fecal samples of herbivore species  $j$  and  $k$ , respectively. For each season, we also calculated dietary separation as the degree of nestedness, and thus deviation from random associations,

in the diets of the herbivore community using the normalized checkerboard score ( $C$ ) (Stone and Roberts 1990). Should the diet of herbivores with a narrower diet form a subset of the diet of species feeding on a wider set of plant taxa, then this will be reflected in high nestedness. Hence, low nestedness will suggest that different herbivores feed on distinct sets of plants. For the stable isotope ratio data, the dietary overlap was calculated as the overlap in the area standard ellipses both within and between species using the R package nicheROVER (Swanson et al. 2015) with default model parameters. For comparison with Schoener's overlap index, we calculated the overall median overlap for pairwise ellipse overlaps, and compared the two measures of dietary overlap using linear regression.

### *Forage quality*

To establish whether the progressing winter was associated with a decline in nitrogen concentration and thus with a decline in forage quality, we examined data on the nitrogen concentration in the fecal samples obtained in the analyses of stable isotopes (Putman 1984; Leslie Jr et al. 2008). To test whether inter-specific variation in nitrogen concentrations within seasons exceeded intra-specific variation, we used analyses of variance (ANOVA).

### *Statistical tests*

~~You could include a paragraph here and present which statistical tests you use to address different questions. [BUT WE DO THAT FOR EVERY QUESTION ABOVE??]~~

## **Results**

All three herbivore species, and in particular Arctic hare and rock ptarmigan, consumed a variety of plant taxa both in early and late winter. Of the trimmed DNA sequence reads, we were able to assign over 99% to plant species, finding no statistical difference in the read

numbers between the replicates or seasons (t-test: t(hare-replicates)=0.02, df=88, P=0.98; t(ptarmigan-replicates)=0.70, df=83, P=0.49; t(muskox-replicates)=0.14, df=71, P=0.89; t(hare-seasons)=0.09, df= 88,P=0.93; t(ptarmigan-seasons)=0.19, df=83 P=0.87; t(muskox-seasons)=-1.00, df=71, P=0.32). Altogether, we identified 46 different plant species or species groups from 22 different families in the sequence data (Appendix S2: Table S2.1) (Fig. 1).

The muskox consumed the lowest number of plant taxa, particularly in late winter (Fig. 1). The diets of all three herbivores in both seasons proved to consist mainly of various shrubs, forbs and graminoids (Fig. 1). The frequencies of occurrence of the various plant taxa in the diets only differed slightly between the two seasons, though generally more plant taxa were consumed in early winter compared to late winter (Fig. 1). Overall, based on the molecular data, the most frequently consumed plant taxa were essentially the same for all three herbivores. Yet, the dietary overlap seemed most pronounced between Arctic hare and rock ptarmigan (Fig. 1). Schoener's index also indicated large overlap between the three herbivores in both seasons (Table 2). For stable isotope ratios, the separation between species was more distinct (Table 2; Fig. 2), although particularly the dietary niche of rock ptarmigan still overlapped considerably with those of Arctic hare and muskox (Table 2). The overlap in dietary niches of Arctic hare and muskox was low in early winter and non-existent in late winter (Table 2; Fig. 2).

Data from the of stable isotope ratios suggested that the Arctic hare and muskox had dietary niches of approximately similar size, while the rock ptarmigan had a much broader dietary niche, particularly in early winter (Table 1; Fig. 2). Within species, the dietary overlap in the two seasons was most pronounced for Arctic hare, intermediate for ptarmigan, and lowest for muskox diets (Table 2). Bootstrapping of overlaps among standard ellipses revealed generally rather narrow confidence intervals for the median overlap, particularly for the inter-specific

comparisons (Appendix S3: Figure S3.1). Schoener's index generally showed the same pattern, but with less variation between seasons (Table 2). Comparing the dietary overlaps obtained from stable isotope ratios corrected versus uncorrected for trophic discrimination factors, revealed qualitatively similar dietary overlaps and confidence limits (Appendix S3: Figure S3.2). The checkerboard scores also indicated increased dietary separation between the three herbivores from early to late winter (early winter:  $C=0.742$ ; late winter:  $C=0.154$ ).

Both Levin's index (based on the molecular data) and the area of the standard ellipses (based on the isotope data) generally showed a consistent pattern of reduced niche breadth as the winter progressed for all three herbivores (**Error! Reference source not found.** 1; Fig. 2). However, Levin's index for rock ptarmigan showed the opposite trend (**Error! Reference source not found.** 1). Despite generally exhibiting the same pattern, the two measures of dietary niche breadth were not significantly correlated with Levin's index ( $R^2=0.01$ ,  $F_{1,7}=0.54$ ,  $P=0.8268$ ), mainly because the relative dietary niche breadth indicated by the two measures were not comparable (**Error! Reference source not found.** 1). Along with general niche contraction, the stable isotope data suggested that the dietary overlap generally declined over winter (Table 2; Fig. 2). This pattern was, however, less evident in Schoener's index, where the overlap was rather consistent in the two seasons (Table 2). Nonetheless, the dietary overlap based on the SEA and the Schoener's index were highly correlated ( $R^2=0.74$ ,  $F_{1,7}=20.26$ ,  $P=0.003$ ).

In terms of forage quality, the nitrogen concentration in the fecal samples was significantly affected by species ( $F_{2,114}=55.45$ ,  $P<0.001$ ), season ( $F_{1,114}=95.60$ ,  $P<0.001$ ) and their interaction ( $F_{2,114}=36.72$ ,  $P<0.001$ ) (full model  $R^2=0.71$ ,  $F_{5,114}=55.99$ ,  $p<0.001$ ; Fig. 3). Tukey post hoc tests revealed that across species, the nitrogen concentrations were significantly lower in early winter compared to late winter ( $p<0.001$ ). The nitrogen concentrations in muskox and rock ptarmigan feces were significantly lower in late winter

compared to early winter ( $P < 0.001$ ), whereas the nitrogen concentration in the diet of Arctic hares was the same in the two seasons ( $P = 0.999$ ) (**Error! Reference source not found.**). In early winter, all three species had fecal nitrogen concentrations significantly different from each other ( $P < 0.001$ ) (highest in rock ptarmigan, intermediate in Arctic hare, and lowest in muskox). In late winter the nitrogen concentrations of rock ptarmigan and muskox feces were not significantly different from each other ( $p = 0.109$ ), while those of Arctic hare differed significantly from the rest ( $p < 0.001$ ) (**Error! Reference source not found.**).

## **Discussion**

Snow challenges the surface-active resident Arctic herbivores by reducing their access to the already sparse food sources in winter, and severe winter conditions may negatively affect the population dynamics of these species (Mech 2000; Hansen et al. 2013; Schmidt et al. 2015). As the growing season comes to an end, the variety and amounts of available forage is markedly reduced compared to summer (Henry et al. 1990; Arndal et al. 2009; Mosbacher et al. 2016a), and the herbivores only have a limited selection of low-nutritious plant forage to choose from during winter. We therefore expected large overlaps in the diets of the three resident herbivores of the Greenlandic High Arctic, particularly in late winter. At this time, the surface-active, resident herbivores are forced to aggregate in increasingly smaller patches of potential foraging areas, where the three herbivore species often forage successively. Due to its larger body size, the muskox is more capable of cratering through more snow to access the plants underneath than both Arctic hare and rock ptarmigan, and may thus facilitate the access to food for these smaller herbivores. Indeed, many of the droppings that we sampled for dietary analyses we found in musk ox craters. Facilitation by musk ox is probably most important in periods with extensive snow cover and deep snow, such as the late winter situation in the present study, where muskox aggregate in less productive areas with less

snow accumulation (Gustine et al. 2011; Schmidt et al. 2016). Indeed, Pedersen et al. (2006) suggested that co-feeding may have evolved to cope with such periods of particularly limited access to food.

Consistent with co-feeding on similar patches of vegetation, the plant taxa found most often in the diets of all three herbivores at Zackenberg were indeed qualitatively similar in both seasons, and each herbivore's diet was comprised of various shrubs, forbs and graminoids. This is consistent with the findings of other dietary studies from the High Arctic, as relying primarily on conventional, morphology based diet analysis (e.g. Thing et al. 1987; Klein and Bay 1991, 1994). Suggesting more differentiation, the area of the standard ellipses of particular rock ptarmigan and the two mammalian herbivores only overlapped slightly (8-10%) in the early winter, while the latter two had even more distinct dietary niches. Hence, although foraging in the very same areas, and despite the limited availability of forage, these three herbivores were seemingly utilizing partly different forage. Contrary to our *a priori* expectations, the overlap declined as winter progressed, and almost no overlap in the ellipse area of any of the three herbivores was observed in late winter. Contrasting with this, the molecular data suggested a high degree of similarity in the diets of the three herbivores in both seasons, though diet was generally less similar in late winter.

Patterns in the overlap of ellipse area are strongly linked to patterns in ellipse width. The ellipse of rock ptarmigan was broadest in early winter, while Arctic hares and muskoxen had smaller and similar-sized dietary niches. In late winter, the dietary niches based on the area of the standard ellipses of all three herbivores were smaller. The shrinkage in dietary niche as winter progressed was also generally supported by Levin's index as calculated for molecular data. However, the relative niche breadth indicated by the two measures showed an inconsistent pattern, and the two measures were not significantly correlated.

Overall, the apparent discrepancies between metrics derived by molecular and isotope data likely reflect the differential information conveyed by each metric. The information derived from stable isotopes is informative regarding quantities consumed (e.g. Kristensen et al. 2011), while the information gleaned from molecular analyses is reduced to presence/absence of plant taxa at the level of individual droppings. Thus, a plant resource consumed frequently but in low quantities by all herbivores will translate into similar resource use, whereas a plant consumed less evenly by individual herbivores but in large quantities whenever encountered will translate into lower overlap. The limited to negligible dietary overlap indicated by the stable isotope data therefore most likely arises from a species- and season-specific consumption, both in terms of species and amounts consumed, but potentially also species-specific intake of specific plant tissues with distinct stable isotope signatures (e.g. Körner et al. 2016).

The nitrogen content in the fecal samples also indicates a difference in plant material used: the rock ptarmigan seem to consume the most nutritious plant material, whereas muskoxen consume the least nutritious plant material. Arctic hare consumed plant material of intermediate nutritional value. For rock ptarmigan and muskox, dietary quality declined from early to late winter, as also previously observed for muskox (Schaefer and Messier 1995). These differences in forage quality are likely rooted in the anatomical differences between the three species, with an increasing ability to digest low-nutrient food from the relative simple digestive tract of the ptarmigan, over the hindgut-fermenting Arctic hare, to the true ruminant, the muskox (Blix 2005).

What the apparent discrepancy between the two methods may thus convey is a consistent imprint of more pre-emptive resource use towards the harsher late winter. With the increasing snow pack, the muskox opening up the crater may consume more of the preferred plants, with the smaller species confined to picking up the scraps of this species, but relying primarily on

other plant species or tissues – each by a mode best suiting its own anatomy and size. In evidence of this, both Arctic hare and rock ptarmigan fed on a much wider range of plant species, even when feeding in the same area as muskox and when operating from the craters opened up by this larger species. Indeed, the plants most frequently consumed by the muskox (*Salix* and *Dryas* in particular; Fig. 1) are dominant species in the vegetation of Zackenberg, and likely the first consumed by a muskox reaching the soil surface through the snow pack.

It thus appears that in the nutrient poor tundra ecosystem, the resident herbivores show some level of differential resource use. These differences are even accentuated in late winter, when snow-cover is almost complete and the snow pack the deepest (Pedersen et al. 2016). Hence, whilst molecular analyses is valuable for detecting the various plant taxa consumed, a combined approach of molecular analyses and stable isotope analyses might be necessary to unravel trophic interactions in more detail (Hardy et al. 2010). We note, however, that dietary overlap between (but not within) species estimated using analyses of stable isotope ratios is still prone to uncertainties due to the trophic discrimination factors applied. As no species-specific factor exist for the three herbivores examined here, we used trophic discrimination factors for closely related taxa as the second-best option, and the dietary overlap presented here is only indicative of true dietary overlap. However, variation in trophic discrimination factors between closely related taxa is small (Caut et al. 2009) and we also found no qualitative changes when contrasting the dietary overlaps estimated from stable isotope ratios corrected vs uncorrected for trophic discrimination factors.

Overall, we therefore conclude that the three resident, snow surface-active herbivore species found in High Arctic Greenland in winter show only limited dietary overlap. A decrease in overlap during the harshest months of the year suggest an increasing specialization on sparse resources. As a likely driver of this specialization, we regard natural selection towards reduced competition. However, the anatomical differences between the three herbivore

species are likely also contributing to the observed pattern. These findings offer cause for concern given ongoing changes in winter conditions and snow regimes in the Arctic (Bokhorst et al. 2016). With changes in snow conditions, and particularly reduced summer soil moisture, winter thaw events and rain-on-snow events (Callaghan et al. 2011; Bokhorst et al. 2016), resource competition may further increase. These patterns were specifically revealed by a combined analysis of molecular data and stable isotope contents. Neither method would have given us the full insight (cf. Hardy et al. 2010), and we strongly encourage the use of the two methods in combination when studying animal diets.

## **Acknowledgements**

We thank the Greenland Ecosystem Monitoring programme for access to ecosystem data, and Aarhus University for the logistic support. Arctic Research Centre, Aarhus University, is thanked for their financial support to the winter field campaign. We thank Aage V Jensen Charity Foundation, the Danish National Research Foundation (CENPERM DNRF100), Jane and Aatos Erkkö Foundation, and the Academy of Finland (grant number 276909) for their financial support. The Finnish Functional Genomics Centre, University of Turku, Åbo Akademi, Biocenter Finland is thanked for the molecular analyses. The authors wish to acknowledge CSC – IT Center for Science, Finland, for computational resources.

## **References**

Adamczewski JZ, Fargey PJ, Laarveld B, Gunn A, Flood PF (1998) The influence of fatness on the likelihood of early-winter pregnancy in muskoxen (*Ovibos moschatus*). *Theriogenology* 50: 605-614.

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215: 403-410.
- Arndal MF, Illeris L, Michelsen A, Albert K, Tamstorf M, Hansen BU (2009) Seasonal Variation in Gross Ecosystem Production, Plant Biomass, and Carbon and Nitrogen Pools in Five High Arctic Vegetation Types. *Arct Antarct Alp Res* 41: 164-173.
- Berg TB, Schmidt NM, Høye TT, Aastrup PJ, Hendrichsen DK, Forchhammer MC, Klein DR (2008) High-Arctic Plant-Herbivore Interactions under Climate Influence. *Adv Ecol Res* 40: 275-298.
- Blix AS (2005) *Arctic Animals and their Adaptations to Life on the Edge*. Tapir Academic Press.
- Boag B, Neilson R, Robinson D, Scrimgeour CM, Handley LL (1997) Wild rabbit host and some parasites show trophic-level relationships for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ : a first report. *Isot Environ Healt S* 33: 81-85.
- Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the Use of Stable Isotopes in Trophic Ecology. *Annu Rev Ecol Evol S* 42: 411-440.
- Bokhorst S, Pedersen SH, Brucker L, Anisimov O, Bjerke JW, Brown RD, Ehrich D, Essery RLH, Heilig A, Ingvander S, Johansson C, Johansson M, Jónsdóttir IS, Inga N, Luoju K, Macelloni G, Mariash H, McLennan D, Rosqvist GN, Sato A, Savela H, Schneebeil M, Sokolov A, Sokratov SA, Terzago S, Vikhamar-Schuler D, Williamson S, Qiu Y, Callaghan TV (2016) Changing Arctic snow cover: A review of recent developments and assessment of future needs for observations, modelling, and impacts. *Ambio* 45: 516-537.

- Callaghan T, Johansson M, Brown R, Groisman P, Labba N, Radionov V, Bradley R, Blangy S, Bulygina O, Christensen T, Colman J, Essery R, Forbes B, Forchhammer M, Golubev V, Honrath R, Juday G, Meshcherskaya A, Phoenix G, Pomeroy J, Rautio A, Robinson D, Schmidt NM, Serreze M, Shevchenko V, Shiklomanov A, Shmakin A, Sköld P, Sturm M, Woo Mk, Wood E (2011) Multiple Effects of Changes in Arctic Snow Cover. *Ambio* 40: 32-45.
- Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors ( $\Delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$ ): the effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol* 46: 443-453.
- Clare EL (2014) Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications. *Evol Appl* 7: 1144-1157.
- Clemmensen KE, Michelsen A, Jonasson S, Shaver GR (2006) Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytol* 171: 391-404.
- Codron D, Sponheimer M, Codron J, Hammer S, Tschuor A, Braun U, Bernasconi S, Clauss M (2012) Tracking the fate of digested  $^{13}\text{C}$  and  $^{15}\text{N}$  compositions along the ruminant gastrointestinal tract: Does digestion influence the relationship between diet and faeces? *Eur J Wildlife Res* 58: 303-313.
- Deagle BE, Thomas AC, Shaffer AK, Trites AW, Jarman SN (2013) Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? *Mol Ecol Resour* 13: 620-633.

- Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26: 2460-2461.
- Elberling B, Tamstorf MP, Michelsen A, Arndal MF, Sigsgaard C, Illeris L, Bay C, Hansen BU, Christensen TR, Hansen ES, Jakobsen BH, Beyens L (2008) Soil and plant community-characteristics and dynamics at Zackenberg. *Adv Ecol Res* 40: 223-248.
- Fogel ML, Cifuentes LA (1993) Isotope fractionation during primary production. In: Engel MH, Macko SA (eds) *Organic geochemistry*. Plenum Press, New York, pp 73-98
- Gray DR (1993) Behavioural adaptations to Arctic winter: shelter seeking by Arctic hare (*Lepus arcticus*). *Arctic* 46: 340-353.
- Gustine DD, Barboza PS, Lawler JP, Arthur SM, Shults BS, Persons K, Adams LG (2011) Characteristics of foraging sites and protein status in wintering muskoxen: insights from isotopes of nitrogen. *Oikos* 120: 1546-1556.
- Hansen BU, Sigsgaard C, Rasmussen L, Cappelen J, Hinkler J, Mernild SH, Petersen D, Tamstorf MP, Rasch M, Hasholt B (2008) Present-Day Climate at Zackenberg. *Adv Ecol Res* 40: 111-149.
- Hansen BB, Grøtan V, Aanes R, Sæther BE, Stien A, Fuglei E, Ims RA, Yoccoz NG, Pedersen ÅØ (2013) Climate Events Synchronize the Dynamics of a Resident Vertebrate Community in the High Arctic. *Science* 339: 313-315.
- Hardy CM, Krull ES, Hartley DM, Oliver RL (2010) Carbon source accounting for fish using combined DNA and stable isotope analyses in a regulated lowland river weir pool. *Mol Ecol* 19: 197-212.

- Henry GHR, Svoboda J, Freedman B (1990) Standing crop and net production of sedge meadows of an ungrazed polar desert oasis. *Can J Bot* 68: 2660-2667.
- Hurlbert SH (1978) The measurement of niche overlap and some relatives. *Ecology* 59: 67-77.
- Ihrmark K, Bödeker ITM, Cruz-Martinez K, Friberg H, Kubartova A, Schenck J, Strid Y, Stenlid J, Brandström-Durling M, Clemmensen KE, Lindahl BD (2012) New primers to amplify the fungal ITS2 region - evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiol Ecol* 82: 666-677.
- Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *J Anim Ecol* 80: 595-602.
- Klein DR, Bay C (1991) Diet selection by vertebrate herbivores in the high arctic of Greenland. *Holarctic Ecol* 14: 152-155.
- Klein DR, Bay C (1994) Resource partitioning by mammalian herbivores in the high Arctic. *Oecologia* 97: 439-450.
- Körner C, Leuzinger S, Riedl S, Siegwolf RT, Streule L (2016) Carbon and nitrogen stable isotope signals for an entire alpine flora, based on herbarium samples. *Alpine Bot* 126: 153-166.
- Krebs CJ (1998) *Ecological methodology*. Benjamin-Collins.
- Kristensen DK, Kristensen E, Forchhammer MC, Michelsen A, Schmidt NM (2011) Arctic herbivore diet can be inferred from stable carbon and nitrogen isotopes in C-3 plants, faeces and wool. *Can J Zool* 89: 892-899.

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88: 42-48.

Leslie Jr DM, Bowyer RT, Jenks JA (2008) Facts from feces: nitrogen still measures up as a nutritional index for mammalian herbivores. *J Wildl Manage* 72: 1420-1433.

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. *EBMnet journal* 17: 10-12.

Mech LD (2000) Lack of reproduction in Muskoxen and Arctic hares caused by early winter? *Arctic* 53: 69-71.

Mosbacher JB, Kristensen DK, Michelsen A, Stelvig M, Schmidt NM (2016a) Quantifying muskox plant biomass removal and spatial relocation of nitrogen in a High Arctic tundra ecosystem. *Arct Antarct Alp Res* 48: 229-240.

Mosbacher JB, Michelsen A, Stelvig M, Hendrichsen DK, Schmidt NM (2016b) Show me your rump hair and I will tell you what you ate - The dietary history of muskoxen (*Ovibos moschatus*) revealed by sequential stable isotope analysis of guard hairs. *Plos One* 11: e0152874.

Norbury GL (1988) Microscopic Analysis of Herbivore Diets - a Problem and a Solution. *Wildl Res* 15: 51-57.

Pedersen AO, Lier M, Routti H, Christiansen HH, Fuglei E (2006) Co-feeding between Svalbard rock ptarmigan (*Lagopus muta hyperborea*) and Svalbard reindeer (*Rangifer tarandus platyrhynchus*). *Arctic* 59: 61-64.

- Pedersen SH, Liston GE, Tamstorf MP, Westergaard-Nielsen A, Schmidt NM (2015) Quantifying episodic snowmelt events in Arctic ecosystems. *Ecosystems* 18: 839-856.
- Pedersen SH, Tamstorf MP, Abermann J, Westergaard-Nielsen A, Lund M, Skov K, Sigsgaard C, Mylius MR, Hansen BU, Liston GE, Schmidt NM (2016) Spatiotemporal characteristics of seasonal snow cover in Northeast Greenland from in situ observations. *Arct Antarct Alp Res* 48: 653-671.
- Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. *Can J Zool* 92: 823-835.
- Pompanon F, Deagle BE, Symondson WO, Brown DS, Jarman SN, Taberlet P (2012) Who is eating what: diet assessment using next generation sequencing. *Mol Ecol* 21: 1931-1950.
- Putman RJ (1984) Facts from faeces. *Mammal Rev* 14: 79-97.
- Roslin T, Majaneva S (2016) The use of DNA barcodes in food web construction - terrestrial and aquatic ecologists unite! *Genome* 59: 603-628.
- Schaefer JA, Messier F (1995) Habitat selection as a hierarchy: The spatial scales of winter foraging by muskoxen. *Ecography* 18: 333-344.
- Schaefer JA, Stevens SD, Messier F (1996) Comparative winter habitat use and associations among herbivores in the High Arctic. *Arctic* 49: 387-391.

- Schmidt NM, Pedersen SH, Mosbacher JB, Hansen LH (2015) Long-term patterns of muskox (*Ovibos moschatus*) demographics in high arctic Greenland. *Polar Biol* 38: 1667-1675.
- Schmidt NM, Berg TB, Forchhammer MC, Hendrichsen DK, Kyhn LA, Meltofte H, Høye TT (2008) Vertebrate Predator--Prey Interactions in a Seasonal Environment. *Adv Ecol Res* 40: 345-370.
- Schmidt NM, van Beest FM, Mosbacher JB, Stelvig M, Hansen LH, Grøndahl C (2016) Ungulate movement in an extreme seasonal environment: Year-round movement patterns of high-arctic muskoxen. *Wildlife Biol* 22: 253-267.
- Smith DM, Grasty RC, Theodosiou NA, Tabin CJ, Nascone-Yoder NM (2000) Evolutionary relationships between the amphibian, avian, and mammalian stomachs. *Evol Dev* 2: 348-359.
- Soininen EM, Gauthier G, Bilodeau F, Berteaux D, Gielly L, Taberlet P, Gussarova G, Bellemain E, Hassel K, Stenøien HK, Epp L, Schrøder-Nielsen A, Brochmann C, Yoccoz NG (2015) Highly Overlapping Winter Diet in Two Sympatric Lemming Species Revealed by DNA Metabarcoding. *Plos One* 10: e0115335.
- Soininen EM, Valentini A, Coissac E, Miquel C, Gielly L, Brochmann C, Brysting AK, Sønstebo JH, Ims RA, Yoccoz NG, Taberlet P (2009) Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. *Front Zool* 6: 16.
- Sponheimer M, Robinson T, Ayliffe L, Passey B, Roeder B, Shipley L, Lopez E, Cerling T, Dearing D, Ehleringer J (2003) An experimental study of carbon-isotope

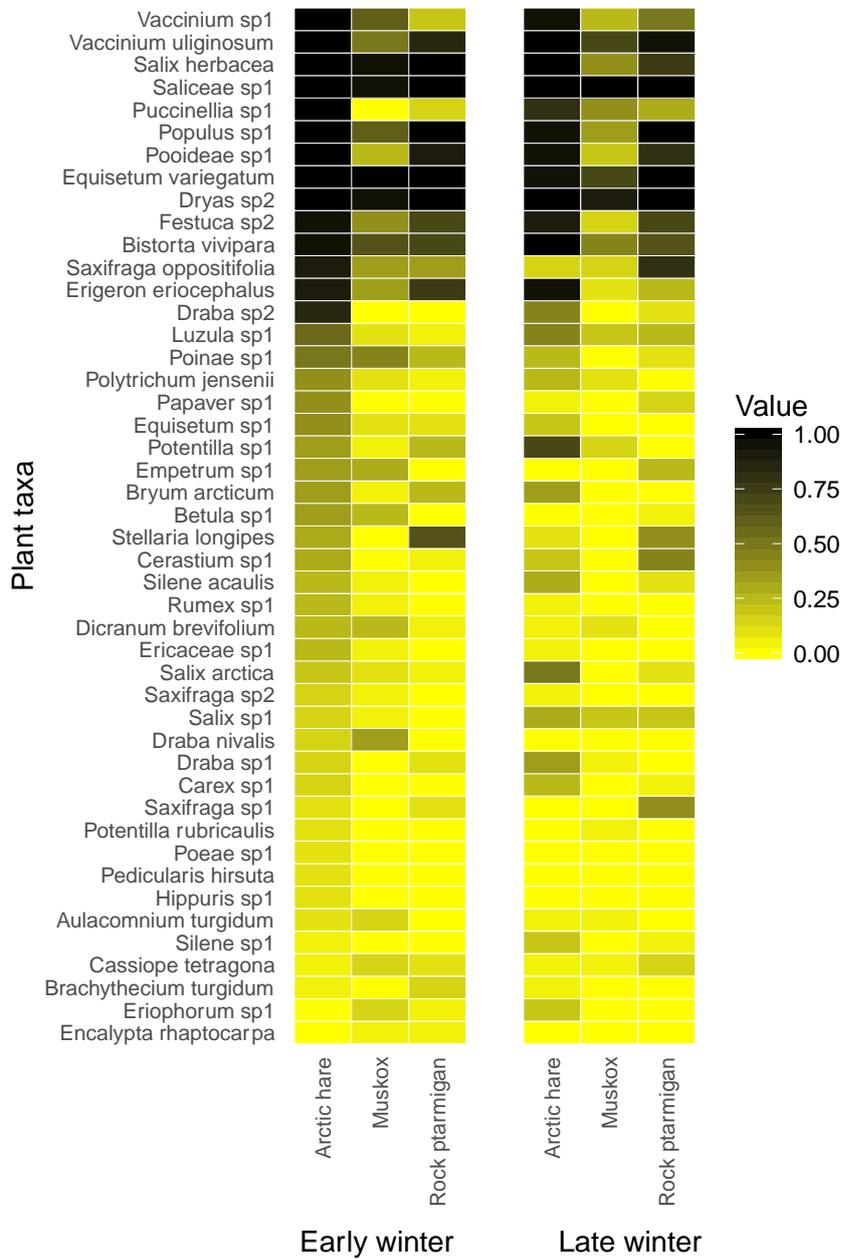
- fractionation between diet, hair, and feces of mammalian herbivores. *Can J Zool* 81: 871-876.
- Stone L, Roberts A (1990) The checkerboard score and species distributions. *Oecologia* 85: 74-79.
- Swanson HK, Lysy M, Power M, Stasko AD, Johnson JD, Reist JD (2015) A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. *Ecology* 96: 318-324.
- Syväranta J, Lensu A, Marjomäki TJ, Oksanen S, Jones RI (2013) An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. *Plos One* 8: e56094.
- Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, Vermet T, Corthier G+, Brochmann C, Willerslev E (2007) Power and limitations of the chloroplast trn L (UAA) intron for plant DNA barcoding. *Nucleic Acids Res* 35: e14.
- Thing H, Klein DR, Jingfors K, Holt S (1987) Ecology of Muskoxen in Jameson Land, Northeast Greenland. *Holarctic Ecol* 10: 95-103.
- Thomas VG (1987) Similar winter energy strategies of grouse, hares and rabbits in northern biomes. *Oikos* 206-212.
- Traugott M, Kamenova S, Ruess L, Seeber J, Plantegenest M (2013) Empirically characterising trophic networks: What emerging DNA-based methods, stable isotope and fatty acid analyses can offer. *Adv Ecol Res* 49: 177-224.

- Vesterinen EJ, Ruokolainen L, Wahlberg N, Peña C, Roslin T, Laine VN, Vasko V, Sääksjärvi IE, Norrdahl K, Lilley TM (2016) What you need is what you eat? Prey selection by the bat *Myotis daubentonii*. *Mol Ecol* 25: 1581-1594.
- White TJ, Bruns T, Lee SJWT, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18: 315-322.
- Willerslev E, Davison J, Moora M, Zobel M, Coissac E, Edwards ME, Lorenzen ED, Vestergard M, Gussarova G, Haile J, Craine J, Gielly L, Boessenkool S, Epp LS, Pearman PB, Cheddadi R, Murray D, Brathen KA, Yoccoz N, Binney H, Cruaud C, Wincker P, Goslar T, Alsos IG, Bellemain E, Brysting AK, Elven R, Sonstebo JH, Murton J, Sher A, Rasmussen M, Ronn R, Mourier T, Cooper A, Austin J, Moller P, Froese D, Zazula G, Pompanon F, Rioux D, Niderkorn V, Tikhonov A, Savvinov G, Roberts RG, MacPhee RDE, Gilbert MT, Kjaer KH, Orlando L, Brochmann C, Taberlet P (2014) Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature* 506: 47-51.
- Wirta H, Várkonyi G, Rasmussen C, Kaartinen R, Schmidt NM, Hebert P, Barták M, Blagoev G, Disney H, Ertl S, Gjelstrup P, Gwiazdowicz D, Huldén J, Ilmonen J, Jakovlev J, Jaschhof M, Kahanpää J, Kankaanpää T, Krogh PH, Labbee R, Lettner C, Michelsen V, Nielsen SA, Nielsen TR, Paasivirta L, Pedersen S, Pohjoismäki J, Salmela J, Vilkkamaa P, Väre H, von Tschirnhaus M, Roslin T (2016) Establishing a community-wide DNA barcode library as a new tool for arctic research. *Mol Ecol Resour* 16: 809-822.

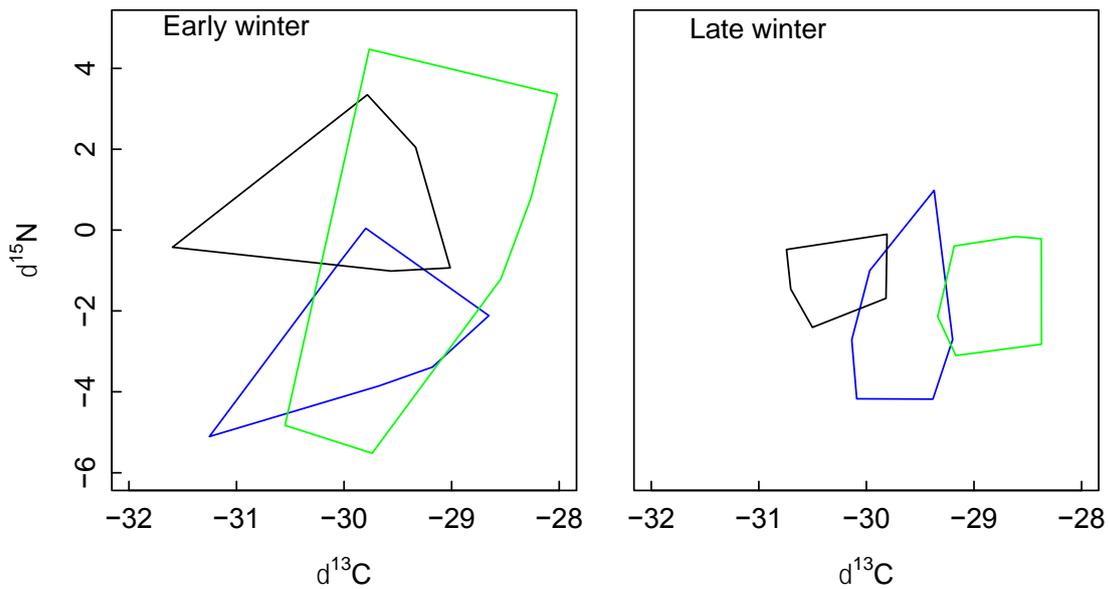
Wirta HK, Hebert PD, Kaartinen R, Prosser SW, Várkonyi G, Roslin T (2014)

Complementary molecular information changes our perception of food web structure. *Proc Natl Acad Sci USA* 111: 1885-1890.

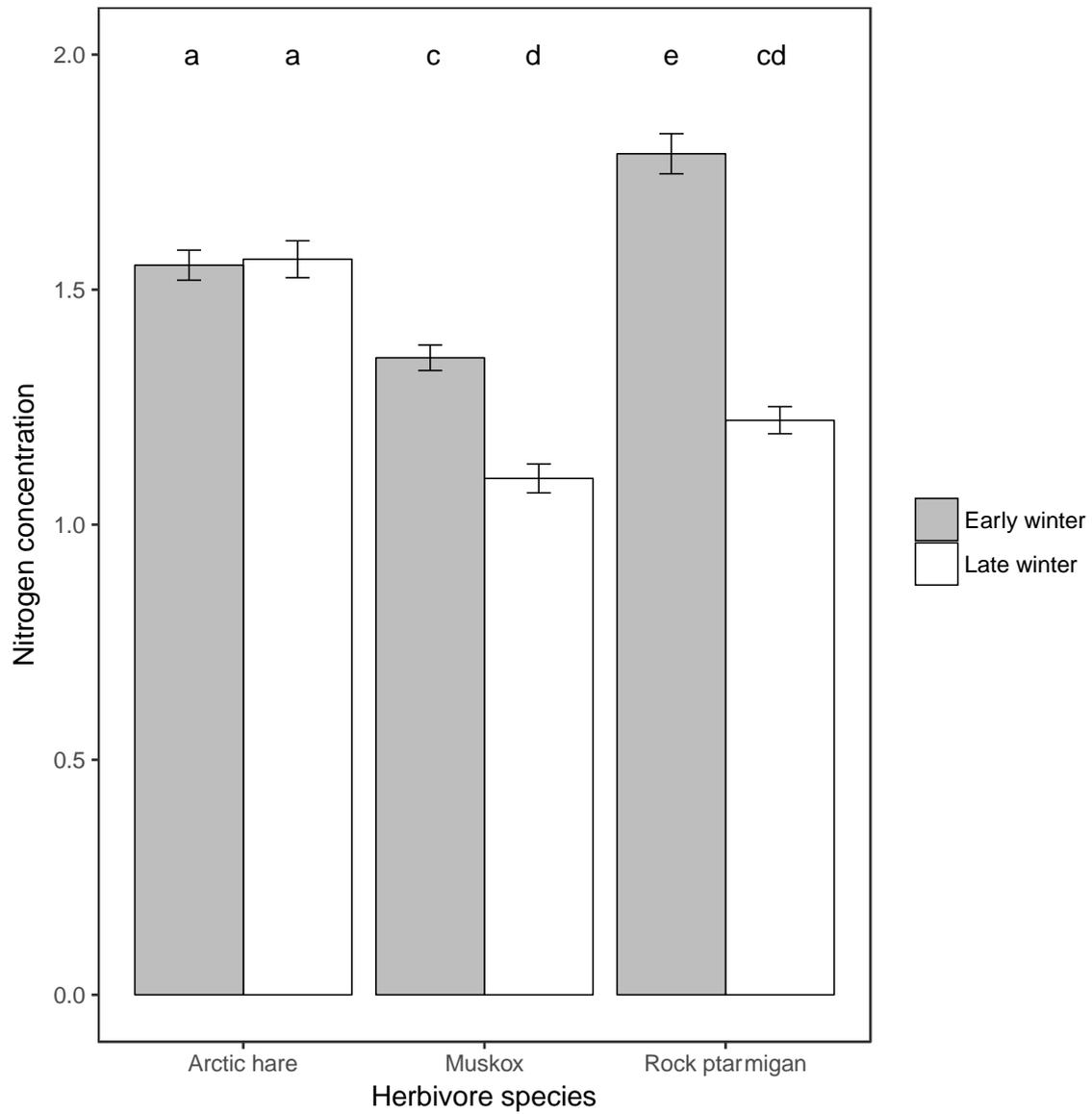
## Figure legends



**Fig. 1.** Heat map showing the frequencies of occurrence in scat samples from the three herbivore species in early and late winter at Zackenberg, Northeast Greenland. The darker the color, the higher the frequency of occurrence.



**Fig. 2.** Dietary niche breadth and dietary overlap amongst three resident herbivores in early and late winter at Zackenberg in Northeast Greenland (Arctic hare (blue), muskox (black) and ptarmigan (green)) as depicted by the standard ellipses based on stable isotope ratios of carbon and nitrogen in fecal samples, as corrected for trophic discrimination factors. The left panel shows early winter, the right panel show late winter. The convex hull is shown here only to illustrate the isotopic dietary niche space of each species.



**Fig. 3.** The mean nitrogen concentration (%) in fecal samples from three resident herbivores species in early and late winter at Zackenberg in Northeast Greenland. Bars indicate standard errors of the mean. Shared letters above bars denote non-significant differences ( $P > 0.05$ ).