Norwegian University
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Linking behavior to diet in Svalbard reindeer (*Rangifer tarandus platyrhynchus*) by use of DNA metabarcoding and GPS-telemetry

Bruk av DNA metabarcoding og GPS-telemetri for å koble bevegelsesatferd til diett for Svalbardrein (*Rangifer tarandus platyrhynchus)*

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Preface

This thesis is the final product of my 2 years in Ås, studying for a master's degree in Nature management in the Norwegian University of Life Sciences (NMBU). Thank to my supervisor professor Leif Egil Loe for great supervising and giving me the opportunity to go to Svalbard for field work. That was truly an unforgettable experience which I really enjoyed. Thank you to Stefaniya Kamenova for help with metabarcoding, and Annie Aasen and Claus Kreibich for help with the Carbon/Nitrogen analysis. Also, I would like to thank Anna-Lena Hendel and Elise Tjørnsletten for great help during field work on Svalbard collecting faeces.

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Abstract

Optimal foraging theory (OFT) provides a framework to link diet quality to behaviour for animals, usually for species where each food items compose a substantial part of the daily energy need, like carnivores or insectivorous birds. Herbivores live in an environment where food usually is abundant and the challenge is to find plants with adequate quality. Herbivore OFT studies are scarce because it is difficult to study their diet and because different aspects influence herbivore behaviour, for example predation-risk, insect harassment or migration.

Svalbard reindeer (*Rangifer tarandus platyrhynchus*) is a particularly suited species to test predictions from OFT on, as they live in an environment without predators, competitors or insect harassment. During the summer of 2018, I sampled 47 faecal samples from 25 different GPS-marked female Svalbard reindeer. I examined their diet quality using Carbon and Nitrogen (C:N) ratio from faeces. I identified plant families in their diet with DNA metabarcoding to find approximate proportions and diversity of plant families. I used step length, turning angle, daily and monthly home ranges derived from GPS-data to relate diet quality to behaviour. To test how diet quality was affected by diet content and behaviour I used likelihood ratio tests of linear mixed models that included individual as a random effect.

I found that individuals differenced significantly in diet quality and then I attempted to explain the individual variation as a function of diet diversity and content and behavioural variables. DNA metabarcoding revealed that the reindeer ate mostly plants from the families Poaceae, Juncaceae, Polygonaceae and Salicaceae. Individual difference in diet quality was not explained by diet diversity, plant families in diet, home range size, movement characteristics, habitat selection or individual attributes such as age and body mass.

Lack of any positive explanations for the individual variation suggest that fine scaled foraging behaviour in bite and patch-selection, below a scale that could be detected with my methodology, might be causing the observed variation in diet quality. My study is the first to report individual differences among female Svalbard reindeer, but the underlying mechanisms are still unknown.

Sammendrag

Optimal furasjeringsteori (engelsk: Optimal foraging theory; OFT) er eit rammeverk av teoriar brukt til å knytte diettkvalitet til adferd hos dyr, vanlegvis for artar som et mat med høgt energiinnhald, som rovdyr eller insektetande fuglar. Planteetarar lev som regel i eit miljø der planter er tilgjengeleg, men utfordringa deira er å finne planter med høg nok kvalitet. Studiar om OFT for planteetarar er det lite av, fordi det er vanskeleg å studere dietten og fordi andre faktorar kan påverke adferden, for eksempel predasjonsrisiko, insektplager eller migrasjon.

Svalbardreinsdyr (*Rangifer tarandus platyrhynchus*) er ein art som er spesielt egna for å teste prediksjonar frå OFT på, fordi dei lev utan rovdyr, konkurrentar eller plagsame insekt. Sommaren 2018 samla eg 47 avføringsprøver frå 25 forskjellige GPS-merka svalbardreinsdyr. Eg undersøkte diettkvaliteten deira ved å bruke forhaldet mellom karbon og nitrogen (C:N ratio) i avføringa. Eg identifiserte plantefamiliane i dietten med DNA-metabarcoding til å finne omtrentlege andelar og diversitet av plantefamiliar. Eg brukte bevegelsesavstand, bevegelsesvinkel, dagleg og månadleg leveområde frå GPS-data til å koble diettkvalitet til adferd. For å sjekke om diettkvalitet var påverka av diettinnhald og adferd brukte eg «likelihood ratio» test av lineære modellar som inkluderte individ som tilfeldig effekt.

Eg fann at individ hadde signifikant forskjell i diettkvalitet og prøvde deretter å forklare denne variasjonen, som følgje av diettdiversitet og bevegelsesadferd. DNA-metabarcoding fann at dietten bestod for det meste av planter frå familiane Poaceae, Juncaceae, Polygonaceae og Salicaceae., men eg fann og stor variasjon i dietten, noko som kan tyder på at dei hadde forskjellige strategiar i næringssøket. I tillegg til data frå metabarcoding, brukte eg GPS-data og prøvde å forklare årsaken til forskjellen i diettkvalitet.

Individuell forskjell i diettkvalitet kunne ikkje forklarast av diversitet i dietten, plantefamiliane i dietten, størrelse på leveområde, bevegelsesadferd, habitatvalg eller individuelle forskjellar. Dette kan indikere at det er forskjellar i delen av planta eit reinsdyr et og korleis dei beitar på finskala nivå, som gjer at dei har forskjellig diettkvalitet. Studien min er den første som finn individuelle forskjellar i diettkvalitet blant simler av Svalbardreinsdyr, men kva mekanismar som fører til denne forskjellen, er framleis ukjent.

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1 Introduction

Optimal foraging theory (OFT) provides a framework to predict animal behaviour when searching for food (Stephens & Krebs, 1986). Concepts of OFT have been used to explain as diverse topics as patrolling police officer behaviour (Sorg et al., 2017) and fishermen's strategies (Begossi, 1992). Most commonly, OFT is used to analyse strategies of maximising energy intake during food search where OFT can predict how animals behave in different environments and with fluctuating food availability (Stephens & Krebs, 1986). OFT can help us understand why animals behaves in a certain way, which in turn can inform management decisions (Bailey et al., 1998; Proffitt et al., 2016). OFT has a wide array of applications: which food types to eat, choice of food patch to feed in, time spent in food patch, optimal foraging speed and movement pattern are all topics investigated under OFT (Pyke et al., 1977). In OFT, optimal food is the one that yields the most energetic value per cost and effort and the dietary breath should be tailored accordingly (Westoby, 1978). A predator always eats the most valuable prey when encountered and only have to include prey of lesser value if the first choice is not available (Hughes, 1979; Estabrook & Dunham, 1976). For example, great tits *(Parus major)* gradually includes prey of lesser value as the preferred food type becomes less available (Krebs et al., 1977). A particularly interesting aspect of OFT is therefore to link search behaviour and degree of selectivity (dietary breath) to the quality of the diet (Owen-Smith, 2002).

Nearly all classical OFT examples are based on species ingesting animal prey and where each food items compose a substantial part of the daily energy need, especially carnivores, insectivorous birds and nectarivores (Senft et al., 1987). OFT studies are much rarer for herbivores (Owen-Smith & Novellie, 1982). Large herbivore animals are mostly generalists (Freeland, 1991), frequently regulated by bottom up food limitation (Côté et al., 2004) and require a different approach when studying OFT. The scarcity of herbivore OFT studies is caused by methodological challenges imposed by their feeding ecology (Stephens & Krebs, 1986). While you can easily observe the prey of a large predator that generally consists of nutritious meat, it is difficult to study both which dietary items are selected as well as the diet quality for free ranging herbivores (Norbury & Sanson, 1992). To observe herbivore diets, microscopy methods using samples from rumen (Bjørkvoll et al., 2009) or faeces (Proffitt et al., 2016) are common. However, there are weaknesses to this method. Small particles are difficult to identify, and plant species can have different levels of digestion (Proffitt et al.,

2016). The manual identification process also requires an experienced person. Recently, metabarcoding has been developed (Taberlet et al., 2012). This is a new and promising tool (Soininen et al., 2009), which uses DNA to identify the species in the diet as well as the approximate proportions (De Barbra et al., 2014; Thomas et al., 2016). Nitrogen (N) is the most used proxy for diet quality for free ranging herbivores, particularly ruminants (Leslie et al., 2008). Parts of plants can have different nutritional value (Van Soest, 1994), therefore knowing plant species is not adequate in the study of herbivore diet. N is crucial to protein synthesis, and a limiting factor for herbivore nutrition (Van Soest, 1994). Faecal N has been found to correlate very well with dietary intake for ruminants (Leslie & Starkey, 1985), and is commonly used a proxy for diet quality (Blanchard et al., 2003; Hodgman et al., 1996). As a continuation, the ratio between Carbon (C) and N in faeces has been used as an inverse proxy for diet quality (Reese et al., 2018; Beumer et al., 2017). C:N ratio is a better expression for N availability (Reese et al., 2018), because percent faecal N does not account for difference in digestibility of plants (Wehausen, 1995; Bernays et al., 1989). Our ability to more accurately study how dietary breadth affect quality in herbivores can be greatly enhanced using a combination of metabarcoding and C:N ratio data.

Search behaviour for animals is expected to pay off in terms of higher food quality (Pyke et al., 1977). Modern GPS (Global Positioning Systems) technology has given us the opportunity to study more fine scaled behaviour of herbivores, as behaviour at different scales can be extracted from GPS locations (Owen-Smith et al., 2010). First, studies have taught us that large scale migration, largely driven by nutritional aspects and especially diet quality (Albon & Langvatn, 1992; Hebblewhite et al., 2008), is common for many herbivores (Fryxell et al., 1988). Second, aspects of the seasonal home range are important, and quality of forage is often correlated with home range size. A smaller home range (from daily to season) usually means higher forage quality (Saïd et al., 2009; Tufto et al., 1996; Bjørneraas et al., 2012; van Beest et al., 2011). Third, food search behaviour often involves moving from one food patch to another. Patches can vary in size and quality, and an herbivore will move to a new patch once the resources are depleted or reaches a certain threshold (Owen-Smith et al., 2010).

In most study systems of large herbivores, behavioural patterns are influenced by other factors than simply selecting for optimal forage (Bailey et al., 1996). Ungulates are often under a constant threat from predators, and effects from predation can mask nutritional limitation (Parker et al., 2009). One criterion in OFT is that foraging must be an independent activity, but this is most often not the case for herbivores (Pierce & Ollason, 1987). For example, the

introduction of wolf (*Canis lupus*) in Yellowstone-national park led to very different habitat use for elk (*Cervus elaphus*), a behaviour that couldn't be explained by altered food availability (Mao et al., 2005). Insect harassment can also influence behaviour, and in southern Norway, wild reindeer (*Rangifer tarandus tarandus*) spent more time moving, thus less time feeding when insect harassment was greater (Hagemoen & Reimers, 2002).

Here I quantify dietary breadth by the use of metabarcoding and searching behaviour from GPS-trajectories and relate it to the diet quality of female Svalbard reindeer *(Rangifer tarandus platyrhynchus)* by using C:N ratio in faeces. The Svalbard reindeer lives in the artic on the archipelago of Svalbard. They are a key actor in a simple terrestrial ecosystem and have no competitors, no predation and minimal insect harassment (Reimers, 1977), also they display negligible migratory behaviour (Tyler, 1987; Tyler & Øritsland, 1989). During the short artic summer, they maximize dietary intake, having no distinct diurnal rhythm (Loe et al., 2007), and stock up energy reserves for the winter, in which they can lose 50% of their total body weight (Reimers et al., 1982; Reimers, 1984). Optimizing foraging behaviour during summer season is therefore important both for survival and reproduction (Albon et al., 2017).

I use 1-4 replicated faecel samples of 25 free-ranging female Svalbard reindeer equipped with GPS transmitters. By having replicates, I will be able to test for systematic differences in diet quality among individuals, and if found, test the following predictions:

1: Diet quality is inversely related to diversity of plant species in faeces, where narrow diets consist of a large portion of species with known high digestibility (notably graminoids).

2: Individuals in low quality areas will need to search more extensively for food, reflected in the home range size. I therefore predict a) that diet quality is negatively correlated to home range-size and b) that individuals with home ranges with a large proportion of graminoids have higher quality diets.

2 Materials and methods

2.1 Study area

Faecal collection was performed from 31st of July till 9th of August 2018 in Nordenskiöldland, Svalbard, approximately 78° north and 15° east. More specifically, the study area consists of the lower parts of Reindalen and the valleys of Colesdalen and Semmeldalen (figure 1).

Figure 1. Map of Svalbard and study area within red markings (TopoSvalbard, 2019).

Climate on Svalbard is cold, with a mean summer temperature usually no more than 5°C (Førland et al., 2011). Precipitation is generally low, with annual precipitation on average less than 200 mm on Svalbard airport (Førland et al., 2011). Lately, due to climate change, temperature and precipitation has increased and is believed to further increase in the future (Førland et al., 2011).

The study area is characterized by large U-shaped valleys with mountain peaks up to 1000m above sea level separating them. Vegetation in the area reaches a height of 250 meter above sea level but is generally sparse, especially in higher altitudes (van der Wal & Stien, 2014). The vegetation is mainly dominated by herbs, graminoids and acidic mires with bryophytes (Elvebakk 2005). Trees and brushes are absent, and vegetation rises usually no more than 5-15 cm from the ground (Hansen 2008). Snow usually covers the landscape from October till June. Plant growth is therefore limited to the period between the beginning of June and lasting till around the start of August (Albon et al., 2017). However, there is large variation within years both in snow cover and plant growth (Karlsen et al., 2014; Albon et al., 2017).

2.2 Study species

Svalbard reindeer is endemic to Svalbard and is the least gregarious Rangifer subspecies, presumably because they don't benefit from living in large groups, due to reduce predation risk (Loe et al., 2007). Instead they live solitary or in small family groups of 2-5 individuals (Reimers, 1977). This might also be a strategy because of the need to tailor the dietary intake to match own body mass and life stage. Svalbard reindeer has denser fur, shorter legs and smaller size than other reindeer species (*rangifer*), all adaptions to a life in the artic (Blix, 2005). Female Svalbard reindeer weigh around 70 kg in the autumn and 50 kg in late winter (Albon et al. 2017).

Svalbard reindeer have to cover at least 74% of their winter energy requirement from forage, despite having a large deposit of fat from the summer (Tyler 1986). The main driver of population dynamics is ground icing events, which block access to forage in winter, while density dependence is less important for regulating the population (Hansen et al., 2014; Albon et al., 2017). While other reindeer species often have a diet dominated by lichens (Staaland et al., 1983), lichens are virtually absent on Svalbard (Beumer et al., 2017). Instead Svalbard reindeer summer diet primarily consists of different grass and sedges, in particular grasses from the Poaceae family (Brattbakk & Øritsland, 1986).

Although Svalbard reindeer is regarded to live in a predator free environment (Loe et al., 2007), occasional predation by polar bear can occur (Derocher et al., 2000) and around 200 reindeer are culled through hunting in certain areas every year (Stien et al., 2012). Despite this, reindeer show little fear for people and seem to quickly habituate to human activity (Colman et al., 2001).

2.3 Data sampling

2.3.1 GPS-data

A total of 40 adult female reindeer were marked with GPS-collars (Vectronic Aerospace, Berlin, Germany) in April 2017 and 2018. All individuals were of known age as they were captured as calves and marked with numbered plastic collars and ear tags. Upon recapture in April, measurements of body mass (weight in kg) and body fat were taken.

Of the 40 GPS-females marked, 25 were used in my thesis. Inclusion was based on whether they were within reach by foot during the summer field period. The collars were scheduled to

obtain a position hourly and transfer all positions over the Iridium satellite network every 4th hour. An assistant sent the most recent locations to my satellite phone and coordinates were plugged into a hand held GPS. Because Svalbard reindeer generally move little over a time scale of a few hours and the landscape is open, the target individuals were nearly always found.

2.3.2 Faeces collection

The focal GPS-marked reindeer were observed and identified from their collar number and ear tag. They were observed from roughly 50-150 meters away with a 15-30x60 Swarovski spotting scope. When the reindeer defecated, one person stayed at the scope, observing the exact position of the faeces, until an assistant retrieved it. VHF walky-talkies were used to communicate and make sure that the correct sample was picked up. Faeces were in most cases found quickly and confirmed to be fresh. In cases of uncertainty, for example a lot of faeces in the area, the sample was discarded, and the process was repeated. Because of this, I feel confident that every sample were from the correct animal. Following sampling, faeces were mixed with similar amounts of silica gel and stored in plastic bags. After the sampling period, samples were kept in -20°C until further analysis. Alongside faecal sample, the reproductive status of the female was also recorded (calf at heel or not). In no case did this vary between observations of the same individual, implying that no loss of calves occurred during the study. However, one individual had uncertain status (a calf in the group could belong to her or another female) and was therefore not included when analysing the effect of calf at heel.

2.3.2 Carbon and Nitrogen analysis

Faeces were separated from silica gel, then dried on 60°C overnight (for minimum 16 hours). The samples were ground and mixed using a Retsch MM400 ball mill (Retsch, Germany) for 3 minutes at frequency 24, which ensured a homogenous sample. Following this, the samples were weighted into thin metal capsules with 5-6 mg of dried faeces, and then analysed for C and N content in an Elementar Vario MICRO cube (Elementar, Hanau, Germany). C:N ratio had a strong negative correlation with nitrogen content in the faeces (Pearson's r = -0.74, $p<0.001$, N=47; figure 2), I therefore chose to continue with C:N ratio as an inverse proxy for diet quality.
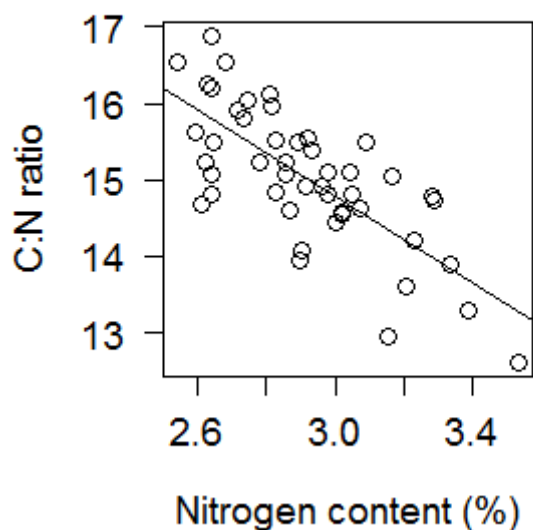
Figure 2. C:N ratio in relation nitrogen content of faecal samples from female Svalbard reindeer summer 2018 (N=47).

2.3.3 Metabarcoding

Molecular diet analysis

Each faecal sample was subsampled prior to C:N analysis by withdrawing 250 mg of wet faeces using disposable lab spatulas (Chemglass, UK). Subsamples were stored in sterile 2-ml microcentrifuge tubes at -20°C prior DNA extraction. DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Germany) according to manufacturer's instructions. Three blank extractions (ultra-pure Milli-Q water instead of DNA) were included for monitoring possible contaminations. DNA amplifications were carried out in a final volume of 15 μL, using the AmpliTaq Gold 360 PCR Master Mix (Thermo Fisher Scientific, USA), 2 μL of DNA extract as template, 0.4 μl/15 ml of BSA and 0.5 μM of each primer. The PCR mixture was denatured at 95°C for 10 min, followed by 35 cycles of 30 s at 95°C and 30 s at the appropriate hybridization temperature for each primer set (appendix 1) and followed by an elongation step for 1 min at 72°C. A 8-9-nt sequence tags were added on the 5' end of each forward and reverse primer, resulting in a unique tag combination for each PCR product in order to allow the assignment of sequence reads for the relevant sample. Each PCR reaction was carried out

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in triplicate and two to three negative) controls (ultra-pure Milli-Q water instead of DNA per 96-well plate were included. One positive control was also included in each 96-well plate with the *gh* primers. Positive controls consisted of artificially assembled mock communities containing a mixture of six unique synthetic DNA stretches mixed at various concentrations (appendix 2). A subset of eight PCR products was randomly selected from each 96-well plate for the visual inspection of the amplified DNA using gel electrophoresis. All PCR products were first pooled per primer set and purified using the QIAquick PCR Purification Kit (Qiagen, Germany). DNA concentration from purified amplicon pools were then quantified using a Qubit™ fluorometer and pooled again prior library preparation and sequencing. Sequencing was carried out on a HiSeq 4000 machine (Illumina, USA), following manufacturer's instructions. A total of 150 nucleotides were sequenced on each extremity of the DNA fragments.

Bioinformatic analyses

Sequences were analyzed using the "OBITools" software (Boyer et al. 2016). First, the direct and reverse reads (corresponding to a single DNA molecule) were aligned and merged using the *illuminapairedend* command by considering the quality of the sequence data during the alignment and the consensus computation. Only alignments with scores >50 were kept for further analyses. Primers and tags were then identified using the *ngsfilter* command. Only sequences with a perfect match on tags and a maximum of two errors on primers were retained for further analyses. Primers and tags were cut off at this step. Strictly identical sequences were clustered together using the *obiuniq* command, while keeping the information about their distribution among samples. All sequences shorter than 10 bp (50 bp for the eukaryote and fungal amplicons) and/or occurring at ≤10 reads, were excluded using the *obigrep* command. Filtered sequences were clustered and spurious sequences removed using *obiclean*. Taxonomic assignations were carried out using the *ecoTag* program (Pegard et al., 2009). *ecoTag* relies on a dynamic programming global alignment algorithm for finding highly similar sequences in a reference database (Needleman and Wunsch 1970). Such databases were built for each primer by extracting the relevant DNA region for eukaryotes, plants, bryophytes and fungi from the European Nucleotide Archive nucleotide library (EMBL, release 136) using the *ecoPCR* program (Bellemain et al., 2010; Ficetola et al., 2010). Finally, a unique taxon was assigned to each sequence with taxa corresponding to the last common ancestor node in the National Center for Biotechnology Information (NCBI)

taxonomic tree of all the taxonomic identifiers (taxIDs) of the sequences of the reference database that matched against the query sequence.

Sequence data filtering

The statistical software R, version 3.5.2 (R Core Team 2018) was used to filter taxonomically assigned sequences for each primer set in order to remove all (i) low-frequency noisy reads, (ii) sequences containing other amplification/sequencing errors, unreliable PCR amplifications or low-quality/low-quantity DNA samples and sequences that were the likely result of contamination or chimeras. The PCR replicates as well as the positive and negative controls was used to adjust filter parameters and evaluate the effectiveness of the sequence analysis process (De Barba et al., 2014).

Diet diversity

Diet diversity was calculated using the Shannon diversity index, which is commonly used as an expression of species diversity in biology (Nolan & Callahan, 2006). It was calculated using the following formula (figure 3), where H is the diversity index, R is the number of families and p is the proportion of all individuals belonging to the *i*th family (Nolan & Callahan, 2006).

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

Figure 3. Shannon diversity index formula used to calculate diet diversity from faecal samples.

2.3.4 Movement analysis

Home ranges were measured using the kernel method from the adehabitatHR package (Calenge, 2011b) in R, Version 3.5.2 (R Core Team 2018), which is known to produce accurate estimates of home ranges (Seaman & Powel, 1996). I used 95% of relocations, in order to exclude the most extreme relocations. Daily home range was defined as 24 hours before collection of the faecal sample. Five faecal samples had unknown time of collection, and for these 15:00 was used for daily home range analysis and daily turning angle and step

length. Monthly home range was defined as 30 days before the first faecal sample was collected for each individual.

Movement data were calculated by using the *ltraj* function in adehabitatLT package in R Version 3.5.2 (R Core Team 2018), that provide a series of useful movement metrics (Calenge, 2011a). Step length is the sum of distance between successive relocations over the entire time period. Relative angle, often called turning angle, is the average angle between successive GPS-relocation. This tells something about animal search behaviour. A value closer to 0 means searching in its close environment, while a value further away from 0 means that an animal searches in a wider area (figure 4; Fletcher & Fortin, 2018).
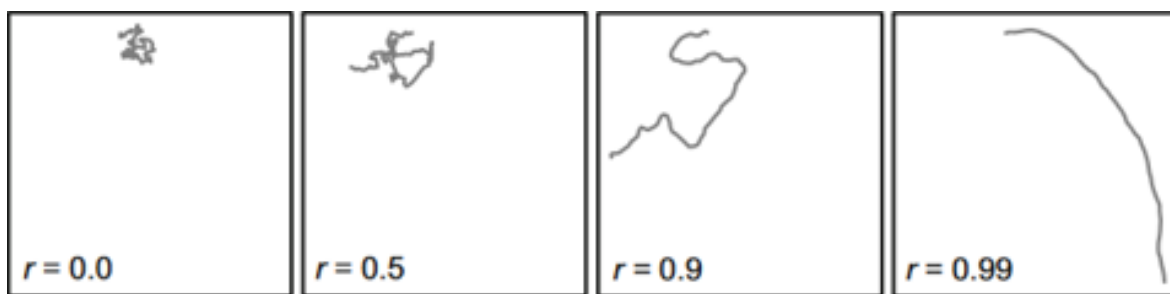

Figure 4. Graphical explanation of turning angle (Fletcher & Fortin, 2018). A value closer to 0 means that an animal is searching in its close environment, while a value firther away from 0 means search behaviour in a wider area.

To calculate vegetation within a home range, a vegetation map from the Norwegian Institute for Nature Research (NINA) was used (Johansen et al., 2012). I used their classification and my interpretation of habitat with a lot of graminoids was map unit number 19 and 20, which I inferred to be good foraging habitat for Svalbard reindeer based on Brattbakk & Øritsland (1986) and references therein. These classes are described as; "*Luxuriant vegetation communities characterised by grasses and forbs combined with a high species number. Associated to warm south- and southwest facing slopes with some supply of water during the growing season.*" (Johansen et al., 2012).

2.4 Statistical analysis

All statistical analysis and graphical presentations were conducted in R, Version 3.5.2 (R Core Team 2018). To check for individual differences in C:N ratio I fitted two models; a

linear mixed effects model with individual as random intercept and a linear model that did not include random effects. Both models were intercept-only models, i.e., no fixed effect predictor variables were included. The two models were subjected to a likelihood ratio test (LRT) (Pinheiro and Bates, 2000) and I concluded that there were significant dietary differences between individuals if the random term was retained in the model with a p-value less than 0.05.

Thereafter, to check for variables explaining C:N ratio, the mixed model with individual as random effect was used as the null model and tested against models with fixed effects. Because of low sample size, only one fixed effect was fitted at a time. Fixed effects that were tested by the use of LRT test were: Diversity of plant families in faeces (Shannon diversity index), proportion of plant families in faeces, home range size, proportion grass inside home range, turning angle, movement distance, April weight, age, backfat in April and calf status. To check for an effect on C:N ratio in faeces from sampling time or sampling date, I fitted a linear model for each, using the *lm* function in R.

3 Results

I sampled faeces from 25 of the focal reindeer in the period from 31st of July 2018 to 9th of August 2018. For 13 out of the 25 individuals I was able to collect replicated samples. In total 47 samples were collected and used for further analysis (table 1). Three of the individuals sampled, did not have an active GPS-collar, but their identity was still known because they had plastic collar and ear tags.

Table 1. Female Svalbard reindeer (ID) used in study with the number of replicated samples of faeces, sampling date, GPS-collar status and age (years) in summer 2018.

ID	Age	Replicates	Gps collar	Sampling date	ID	Age	Replicates	Gps collar	Sampling date
B135	9	2	no	6.7. Aug	R320	6	2	yes	2.3. Aug
B139	9	1	no	4. Aug	W106	8	1	yes	1. Aug
B158	9	1	no	6. Aug	W127	8	3	yes	5.6.7. Aug
G117	7	3	yes	1.2.3. Aug	W128	8	1	yes	7. Aug
G118	7	2	yes	6.7. Aug	W137	8	2	yes	6.7. Aug
G120	7	3	yes	5.6.7. Aug	W138	8	3	yes	1.2.3. Aug
G141	7	2	yes	6.7. Aug	W139	8	3	yes	5.6.8. Aug
R289	7	1	yes	3. Aug	W150	8	3	yes	31. Jul 1.2. Aug
R290	7	1	yes	3. Aug	Y136	5	1	yes	4. Aug
R297	6	1	yes	9. Aug	Y137	5	4	yes	31. Jul 1.2.4. Aug
R310	6	1	yes	4. Aug	Y147	5	1	yes	6. Aug
R312	6	1	yes	7. Aug	Y205	5	1	yes	7. Aug
R318	6	3	yes	1.3.4. Aug					

3.1 Plant families in diet

The contents of the faeces had plants from 28 different plant families represented (table 2). Plants from the *Poaceae* family were on average the most common species found in the samples, with an average of 35,36% in all samples. There was great variation in the samples, one sample had 93% plants from the *Cyperaceae* family while it was not found in others.

Table 2. Average, maximum and minimum proportions of plant families found in the faecal samples of the 25 female Svalbard reindeer in summer 2018 (N=47).

Plant family	Average	Maximum	Minimum	Plant family	Average	Maximum	Minimum
Amblystegiaceae	0,01 %	0,23 %	0,00 %	*Juncaceae*	18,84 %	59,00 %	0,17 %
Anacardiaceae	0,00 %	0,00 %	0,00 %	*Orobanchaceae*	0,01 %	0,10 %	0,00 %
Athyriaceae	0,00 %	0,01 %	0,00 %	*Papaveraceae*	0,04 %	0,52 %	0,00 %
Aulacomniaceae	0,00 %	0,02 %	0,00 %	*Poaceae*	35,36 %	69,34 %	2,57 %
Bartramiaceae	0,00 %	0,00 %	0,00 %	*Polemoniaceae*	0,00 %	0,02 %	0,00 %
Betulaceae	0,22 %	9,93 %	0,00 %	*Polygonaceae*	13,92 %	55,33 %	0,61 %
Brassicaceae	0,13 %	2,71 %	0,00 %	*Polytrichaceae*	0,03 %	0,42 %	0,00 %
Bryaceae	0,05 %	0,52 %	0,00 %	*Ranunculaceae*	1,05 %	11,71 %	0,00 %
Campanulaceae	0,00 %	0,04 %	0,00 %	*Rhabdoweisiaceae*	0,00 %	0,01 %	0,00 %
Caryophyllaceae	1,43 %	24,64 %	0,00 %	*Rosaceae*	0,01 %	0,28 %	0,00 %
Closteriaceaee	0,00 %	0,02 %	0,00 %	*Salicaceae*	14,12 %	44,55 %	0,29 %
Cyperaceae	10,37 %	93,44 %	0,00 %	*Sapindaceae*	0,00 %	0,00 %	0,00 %
Desmidiaceae	0,00 %	0,11 %	0,00 %	*Saxifragaceae*	4,24 %	33,49 %	0,00 %
Equisetaceae	0,06 %	0,43 %	0,00 %	*Sphagnaceae*	0,00 %	0,08 %	0,00 %
Fabaceae	0,00 %	0,03 %	0,00 %	*Splachnaceae*	0,11 %	1,16 %	0,00 %
Grimmiaceae	0,00 %	0,00 %	0,00 %	*Timmiaceae*	0,00 %	0,01 %	0,00 %
Hylocomiaceae	0,00 %	0,00 %	0,00 %				

3.2 Diet quality

Among the 25 female reindeer, there was a significant individual difference in faecal C:N ratio (inverse proxy for diet quality) (p=0.002; figure 5), enabling a search for factors causing individual difference in diet quality.
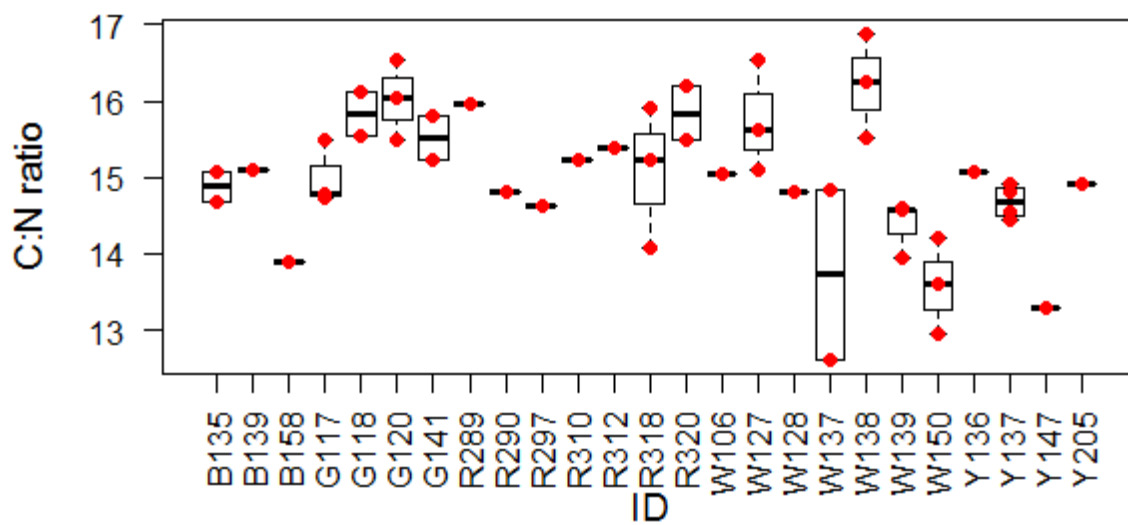

Figure 5. Faecal C:N ratio in summer 2018 for the 25 female Svalbard reindeer (N=47).

3.3 Relationships between diversity and quality of the diet

No correlation was found between diet quality (C:N ratio) and Shannon diversity of the diet (estimate=0.19, SE=0.43, p=0.67) (figure 6). This does not support my first prediction, which predicted better diet quality (lower C:N ratio) at low diversity.
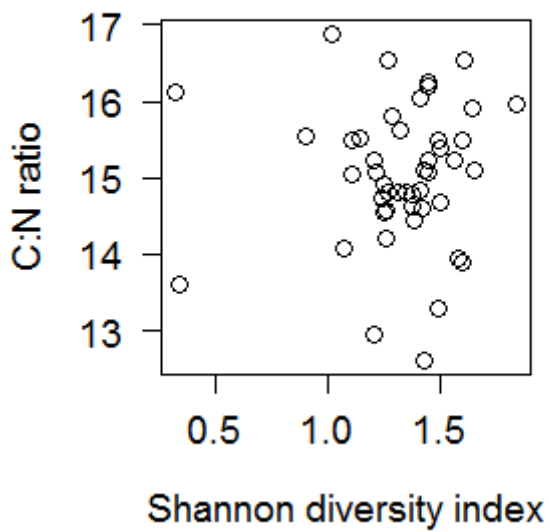
Figure 6. Faecal C:N ratio as a function of faecal plant family diversity (Shannon diversity index) for the 25 female Svalbard reindeer in summer 2018 (N=47).

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3.4 Relationship with plant families in faeces and diet quality

Rejecting my second prediction, the proportional content (%) of Poaceae nor any of the other
3 most predominant plant families in faeces did not correlate with faecal C:N ratio (figure 7)
(Poaceae: estimate=0.002, SE=0.008, p=0.79; Juncaceae: estimate=-0.0032, SE=0.0073,
p=0.65; Salicaceae: estimate=-0.0084, SE=0.008, p=0.4 1 and Polygonaceae: estimate=0.003,
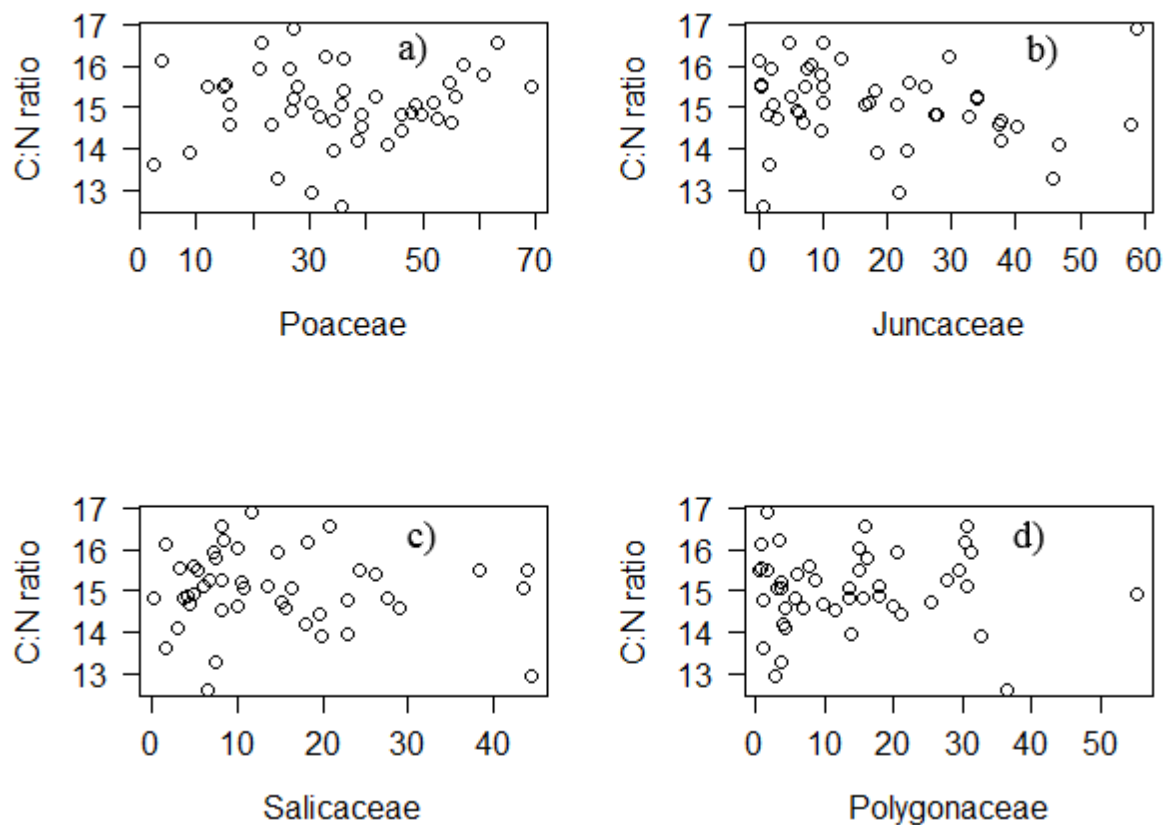SE=0.0103, p=0.77).

Figure 7. Faecal C:N ratio as a function of proportion (%) of the plant families Poaceae (a), Juncaceae
(b), Salicaceae (c) and Polygonaceae (d) found in faecal samples from the 25 Svalbard reindeer in
summer 2018 (N=47).

3.5 Effects of home range and movement characteristics on diet quality

A total of 43 out of the 47 samples from known individuals were from animals with GPS-data available (table 1). Difference in C:N ratio could not be explained by the home range size (km^2) for either the a) daily (estimate=0.008, SE=0.18, p=0.94) or b) monthly home range (estimate= -0.00012, SE=0.00029, p=0.66) (figure 8). This was contrary to my prediction (2a).
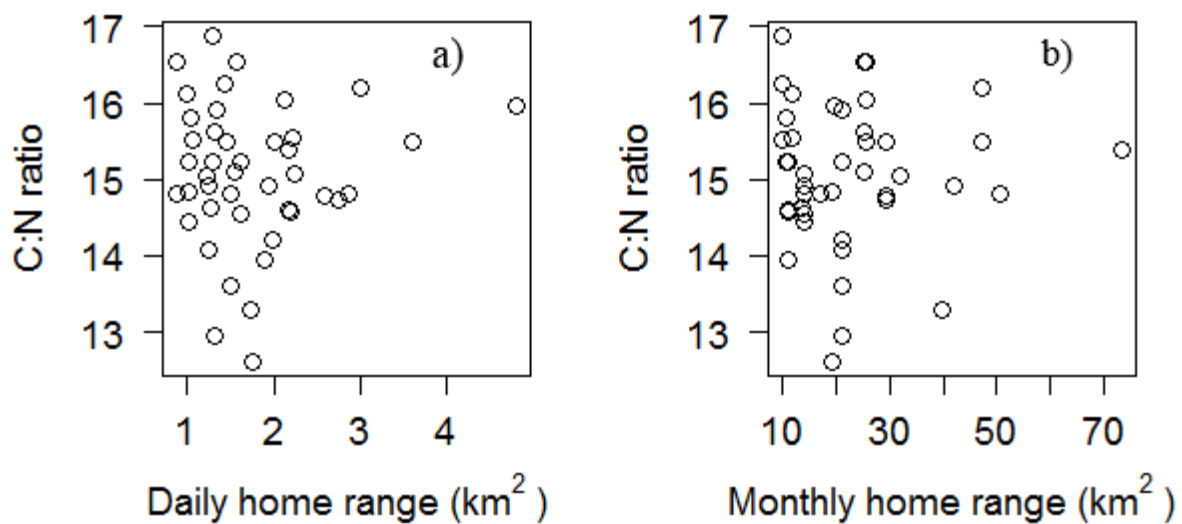

Figure 8. Faecal C:N ratio as a function of a) daily home range size (km^2) and b) monthly home range size (km^2) for Svalbard reindeer in summer 2018 (N=22 individuals, N=43 faecal samples).

3.6 Habitat selection in relation to diet quality

Proportion of grass inside daily home range (%) showed a trend to increase C:N ratio (decrease diet quality), but only for daily home range (estimate=0.021, SE=0.011, p=0.08; figure 9a). In the monthly home range no such trend was found (estimate=0.0071, SE=0.018, p=0.69; figure 9b). Again, lack of expected relationship was contrary to my prediction (2b).
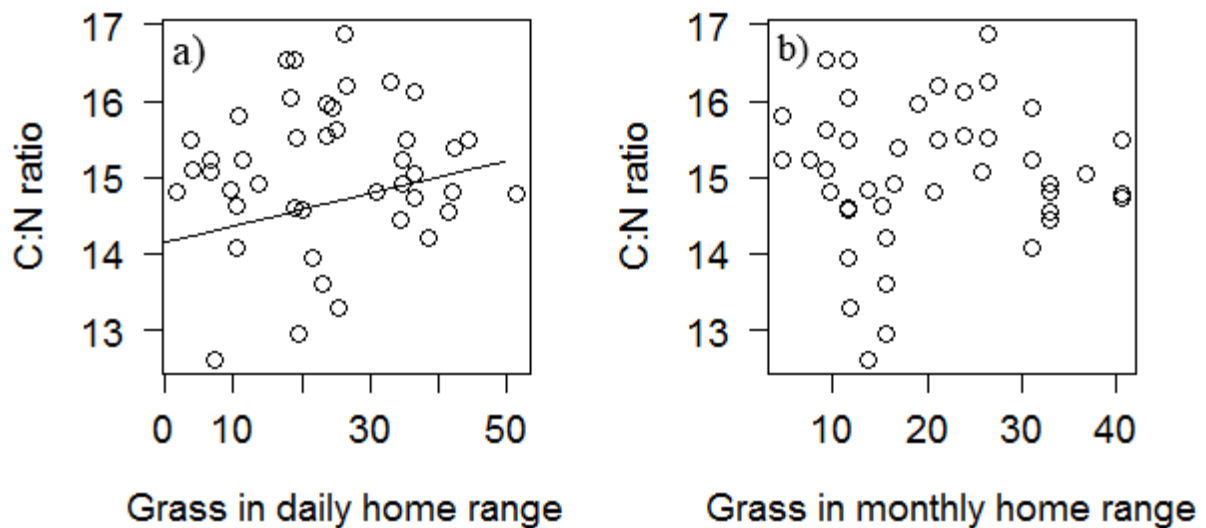

Figure 9. Faecal C:N ratio as a function of proportion of grass inside home ranges (%), both daily (a) and monthly (b), of GPS-marked female Svalbard reindeer (N=22 individuals, N=43 faecal samples).

3.7 Turning angle and movement distance in relation to diet quality

Daily turning angle did not affect C:N ratio in faeces (estimate= -0.20, SE= 0.38 p=0.6; figure 10a), neither did monthly turning angle (estimate=1.91, SE=3.51 p=0.57; figure 10b), total daily step length (estimate=-0.014, SE=0.11 p=0.92; figure 10c) or total monthly step length (estimate= 0.006, SE= 0.014 p=0.67; figure 10d). This was not as predicted by my prediction that search behaviour reflect diet quality for Svalbard reindeer.
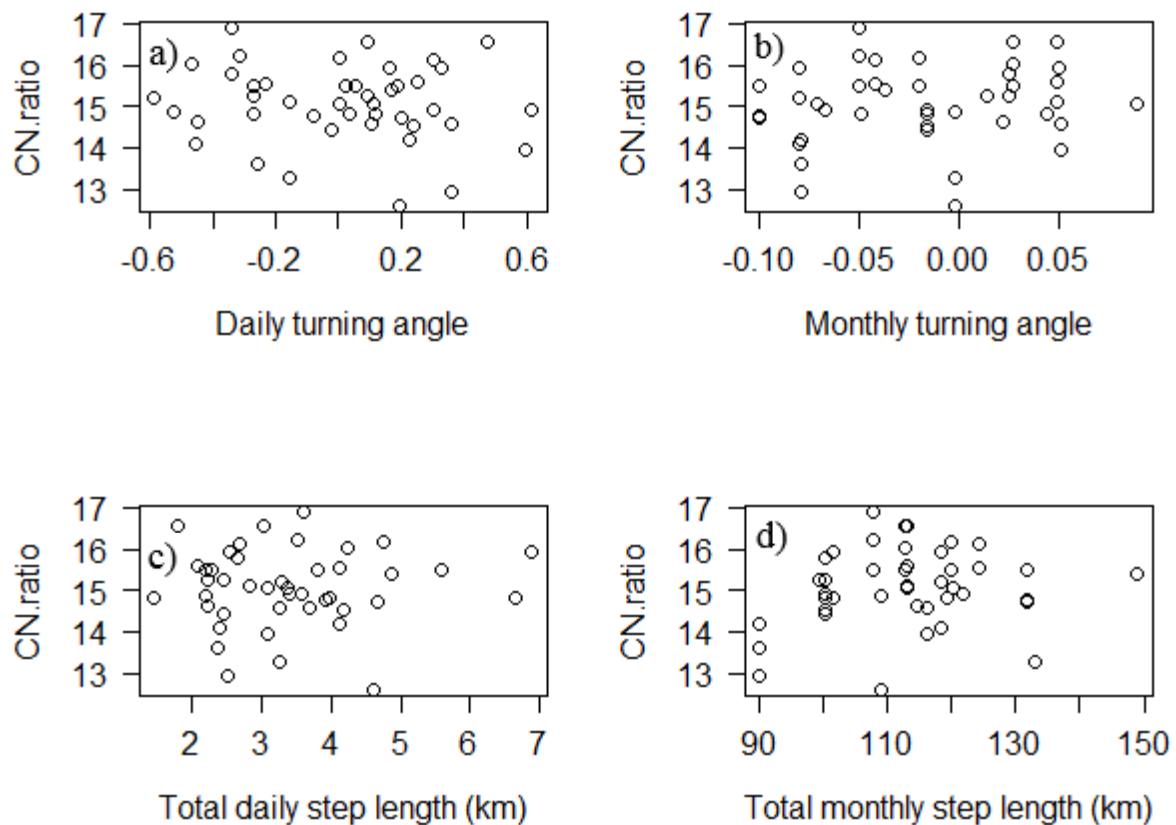

Figure 10. Faecal C:N ratio as a function of average daily turning angle (a), average monthly turning angle (b), total daily step length (c) and total monthly step length (d) for Svalbard reindeer during summer 2018 (N=22 individuals, N=43 faecal samples).

3.8 Relationships between individual factors and diet quality

Individual factors had in general no effect on diet quality, neither individual age (estimate=-0.02, SE=0.13, p=0.83, p=0.12 for the second order effect; figure 11a) nor backfat (estimate=0.015, SE=0.03, p=0.6; figure 11b). While 17 females had a calf at heel, 7 females were barren or had lost the calf earlier in the season. No difference in C:N ratio was found between these two groups (estimate= -0.24, SE=0.37, p=0.5; figure 11c). Surprisingly, reindeer with low and high April weight had significantly better diet quality than the ones from 53-57 kg (p=0.014 for the second order effect; figure 11d).
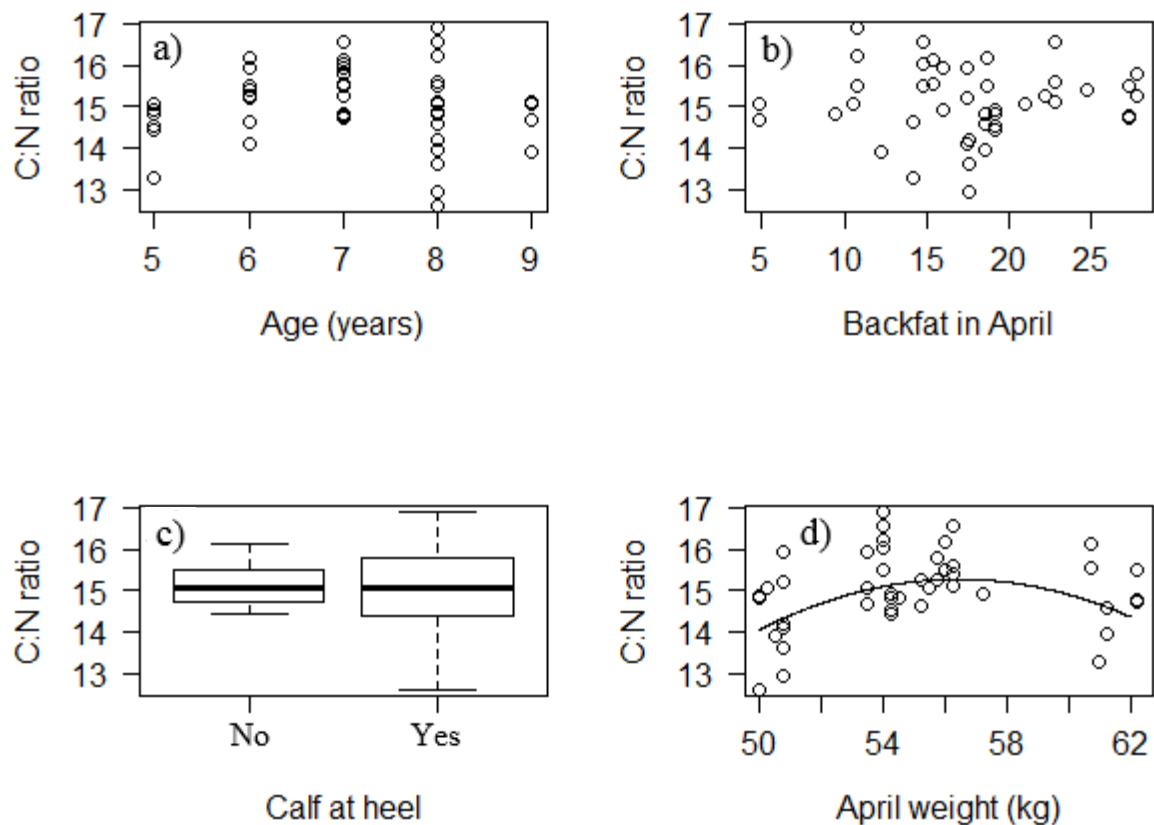
Figure 11. Faecal C:N ratio in relation to a) age (N=25 individuals, N=47 faecal samples), b) backfat in April (N=23 individuals, N=44 faecal samples), c) calf at heel or not (N=24 individuals, N=46 faecal samples) and d) April weight (kg; N=24 individuals, N=46 faecal samples) for the female Svalbard reindeer in the summer 2018.

3.9 Effect from sampling time and date on diet quality

Faecal samples was collected throughout the day, with the earliest sampled at 9:00, while the three latest at 20:00, but there was no effect of daily sampling time on C:N ratio in faeces (estimate=0.054, SE=0.056, p=0.34; N=42; figure 12a). The first two samples were sampled on 31st of July, and the last sample at 9th august, but also sampling date did not affect faecal C:N ratio (estimate=0.02, SE=0.06, p=0.7; N=47; figure 12b).

Figure 12. Faecal C:N ratio in samples collected from female Svalbard reindeer summer 2018 in relation to a) sampling hour (N=42) and b) sampling date (N=47).

4 Discussion

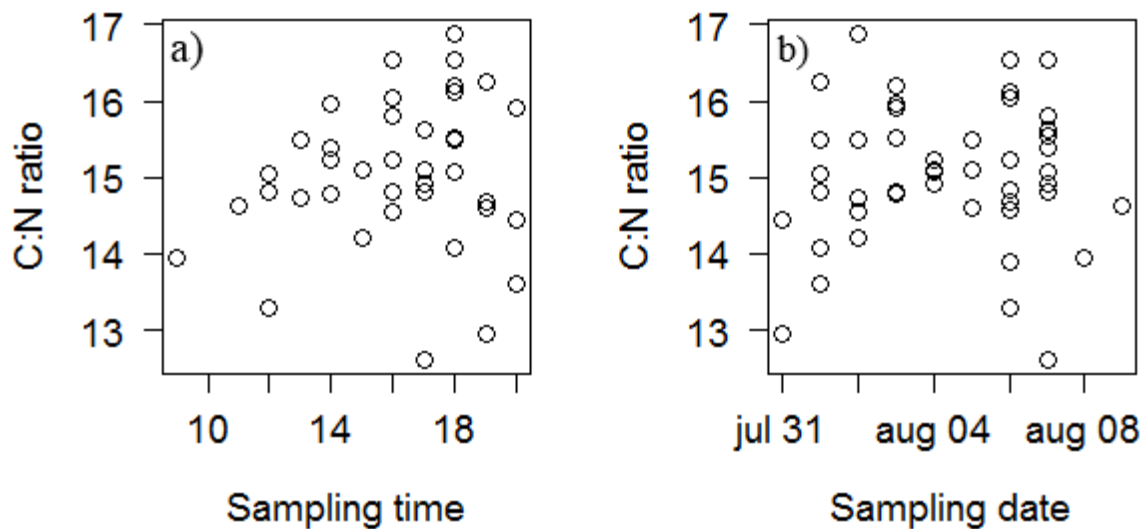I tested predictions from OFT in order to test factors affecting diet quality in Svalbard reindeer. My study is the first to detect systematic individual differences in diet quality of Svalbard reindeer, but neither of the tested characteristics of diet, movement nor individual traits could explain this individual difference in diet quality. There are several possibilities for this lack of relationships. First, arctic vegetation is of generally high quality with low levels of secondary compounds, probably due to the short growth season (Staaland, 1984). Indeed, in a previous study Svalbard reindeer have been shown to select for quantity rather than quality of vegetation (van der Wal et al., 2000), which will weaken the expectation of an inverse relationship between dietary niche breath and food quality. With respect to missing effects of behaviour it is likely that feeding behaviour at much smaller scales than the home range and on shorter time scales than hours affect feeding efficiency and diet quality. While my results show systematic differences in individuals' ability to obtain a high-quality diet, the underlying mechanisms are still unknown.

4.1 Dietary contents as diet quality proxy

Large herbivores take advantage of a generally available resource and experience a large variation in diet quality. Their challenge is often not the quantity, but the quality of food (Van Soest, 1994). Ruminants also gain more energy and spend less time ruminating when eating high quality forage, therefore being very selective in which species they ingest (White, 1983). My first prediction was therefore that dietary breadth was inversely correlated with diet quality, but this got no support in my results. Westoby (1978) argues that a diet consisting of few species is not always the optimal diet for a herbivore, which according to him cannot just eat one plant to survive, like predators or insects. In addition, Wang et al. (2010) found that sheep (*Ovis aries*) preferred a diverse diet, even though the most palatable species were available. Others have also argued that herbivores need to eat diverse due to limited detoxification ability of defence compounds found in plants (Dearing et al., 2000). My findings suggest that a narrow diet neither cause a high- nor a low quality diet, which means that degree of selectivity on the plant species level could be less important in Svalbard reindeer than in many other species.

Along the same line, I found no relationship between the proportion of any of the main plant families and diet quality, which was unexpected, as different plants have different nutritional value for ruminants (Van Soest, 1994). A relationship with species in diet and diet quality has been found in other studies (Redjadj et al., 2014), for example Wang et al. (2018) that found different diet quality (C:N ratio) between sheep (*Ovis aries*) and cattle (*Bos taurus*) and could partly explain this due to different forage species. In my study, reindeer ate plant families known to be of high quality, particularly Poaceae (Staaland, 1984). Lack of a relationship between the proportion of Poaceae (that varied across individuals from 2,57 to 69,34 %) and diet quality is surprising. However, van der Wal et al. (2000) suggest that plant quality is not a limitation for Svalbard reindeer, and they select for quantity instead. Plants on Svalbard are of generally high quality and are highly digestible (Staaland, 1984; Staaland et al., 1983). Quality of forage is therefore probably not of great concern for the Svalbard reindeer and can be met by a range of different plant species. The large individual difference in dominant plant families in the faeces indeed suggests that Svalbard reindeer may have different foraging strategies.

4.2 Forage selection

Food selection for herbivores is dependent on scale (table 3; Owen-Smith, 2010; Senft et al., 1987). They often select for quantity on food on landscape level, but for quality on a finer scale (van Beest et al., 2010; Kaszta el al., 2016). I found no signs that Svalbard reindeer selected for quality on home range scale, meaning diet quality selection probably took place on a finer scale. The direct cause for diet quality is a result from what is clipped when an herbivore eats (Fortelius, 1985), but my methods would not have been able to detect difference in bite size or which part of the plant is clipped. However, lack of positive findings suggests that selection on small scale including plant parts may be important.

Table 3. Forage selection of different scales for herbivores (Owen-Smith, 2010).

temporal scale	spatial scale	defining behaviour	vegetation unit
1–2 s	bite	plucking, chewing and swallowing	plant part
2 s–2 min	feeding station	moving head, prehending, biting	plant (grass tuft, shrub)
0.5–30 min	food patch	feeding (eating), stepping	clump of plants
1–4 h	foraging area	feeding, walking, standing alert	habitat patch
12–24 h	daily range	foraging, travelling, drinking, ruminating, resting	set of habitats
3–12 months	home range	growth, reproduction, mortality	landscape region
several years	lifetime range	survivorship, fecundity, dispersal	geographical region

4.3 Home range size and movement behaviour

A home range must satisfy the required need for energy and nutrition, or it must be increased (Saïd et al., 2009; van Beest et al., 2010). Also, when a foraging animal enters a patch with high quality forage, step length is expected to decrease, and the trajectory will be more tortuous (De Knegt et al., 2007). To the contrary, I found neither an effect of home range size (daily or monthly), movement distance, nor turning angles on diet quality. A possible explanation is that there is not enough spatial variation in forage quality at the scales resembling a typical Svalbard reindeer home range, implying that both individual with large and small home ranges to the same extent met their feeding requirements. As Svalbard reindeer to a large extent forage while walking, moving between foraging patches on a scale of meters or tens of meters, the selected route may be more important than the size of the home range in terms of foraging efficiency. Also, if Svalbard reindeer simply enlarge their home range if their diet quality is not adequate, it would mask effect from home range size on diet quality. In addition, other factors may be more important for variation in home range size, for example age, body size or reproductive status (van Beest et al., 2011).

4.4 Habitat selection in relation to diet quality

According to Stephens & Krebs (1986), herbivores choose foraging spot, instead of foraging species, because of the stationary nature of their food. Compared to predators large, long-lived herbivores can be expected to have a more precise knowledge of distribution of food within their home range because it is predictable. I expected that a larger proportion of grass inside a home range would lead to an increase in diet quality, as more food abundance could present an opportunity for the reindeer to be more selective, both in which species and part of plants to digest. Instead, the proportion grass inside daily home ranges tended to correlate negatively with diet quality. This was surprising, and contrary to my prediction. Mårell et al. (2002) found that semi-domesticated reindeer in Sweden selected for feeding sites with more biomass, but they found no selection for N content in plants within those patches. Selection for a grass rich diet may therefore not be reflected in higher N. Canon et al. (1987) found that free-ranging tame elk (*Cervus elaphus nelsoni*) preferred to feed in burnt sites in a aspen forest, compared areas untouched by recent fire, but also found no difference in nutritional diet. However, elk that foraged in the burnt areas spent significantly less time feeding than the

others (i.e., they were time minimizers rather than energy maximisers; Bergman et al., 2001). Still, time minimizing is expected only in species subjected to predation and not in Svalbard reindeer where deploying a fat store is expected to be the main driver for behavioural decisions during summer. My results further suggest that diet quality is not a main concern for Svalbard reindeer.

The trend of a positive relationship between proportion of grass within the daily home range conflicted with the lack of such a relationship between proportion of grass and C:N ratio in the faeces. Reese et al. (2018) assessed grass consumption and found a positive correlation to C:N ratio among 30 different mammals in east Africa. This was the same trend as I detected on the home range scale. He attributed his result to different physiological features among species, and included not only ruminants, but also both omnivores and hindgut fermenters, therefore not very applicable to my study. I suspect my findings could be a random effect, since it was only a trend, and the more direct relationship between proportion of graminoids and quality in the faecal data found no such relationship.

4.5 Effect from individual differences on diet quality

Individual traits, like sex, lactation or body size can affect diet quality in ungulates (Monteith et al., 2014; Demment & Van Soest, 1985). In my study, age, amount of body fat in April and calf at heel were tested but found to not affect diet quality. Surprisingly, April weight correlated with diet quality. This pattern has not been reported by others and is not an expected from any known biological mechanism. Therefore, I expect this is only a random effect caused by the small sample size. Contrary to my study, a lower amount of N in faeces has been found in lactating females compared to non-lactating females of white-tailed deer (*Odocoileus virginianus*) (Monteith et al., 2014). This result also supports the notion that forage is at generally good quality on Svalbard, and that diet quality is not a limiting factor for neither reproductive nor non-reproductive Svalbard reindeer. My study was conducted about 2.5 months after calving, at a time when they are highly mobile and do not restrict the ranging behaviour of their mother.

4.6 Factors hypothesized to explain the unexplained individual variation in diet quality

Tooth wear, and differences in teeth efficiency can affect particle size, in turn affecting diet quality in ungulates (Fortelius, 1985). For Svalbard reindeer tooth wear correlates to particle size in rumen (Veiberg et al., 2007), which could possible reduce digestion and increase faecal C:N ratio. Although I don't have any measurements for teeth in my data set, particle size in rumen has found to be closely correlated to age and weight for Svalbard reindeer (Veiberg et al., 2007). Therefore, the lack of an effect of age and the surprising curvilinear effect of mass, suggests that tooth wear is not an important explanatory factor of diet quality in my study. The width of the incisor arcade is another ruminant trait that could potentially cause variation in individuals' ability to be selective in grazing (Gordon & Illius, 1988). Variation in bite mass is closely positively correlated to this metric across species (Gordon et al., 1996). Reindeer have smaller bite size than sheep and cattle, but higher bite rate, suggesting that reindeer can be more selective (Trudell & White, 1981). However, Hardenberg et al. (2003) found no signs that incisor arcade size for bighorn sheep (*Ovis canadensis*) was a direct effect of selection, but rather due to age and body size. I regard it unlikely that individual variation within one species and one sex is sufficient to cause large variation in selectivity and diet quality.

Other herbivores commonly have a day/night cycle where they eat and rest at certain times during the day (Schoener, 1971). In such a system, my sampling time could have affected the results. Svalbard reindeer doesn't have a distinct pattern of day/night cycle during summer (Loe et al., 2007), therefore I didn't expect diet quality to be affected by this. This was also what I found. Likewise, date had no effect on diet quality. Plant quality degrades as the growing season goes by (van der Wal et al., 2000), and this caused a deterioration in diet quality for domestic free ranging sheep *(Ovis aries)* in Norway (Mysterud et al., 2011). My study was done in a short time span which probably explain why no effects of degrading plant quality was found.

4.7 Applicability of OFT for large herbivore mammals

The applicability for OFT in real life scenarios has been extensively debated (Sih & Christensen, 2001; Pierce & Ollason. 1987), and Hanley (1997) doubts that animals can accurately identify forage value and behave in a way that makes the OFT realistic. He thinks

learnt behaviour is highly complex, and an "optimal" solution might be a scientific solution, but in real life a "good" solution seems more likely. OFT predicts that an animal responds with an increased eating rate when forage quality is poorer (Pyke et al., 1977), but Sinclair et al. (1982) indicated that snow-shoe hare was unable to act accordingly, as they ate at a constant rate, even when given plants with poorer quality. My results indicate that Svalbard reindeer apply different foraging strategies, due to the varied contents in faeces, but if this is a conscious choice by reindeer or due to random occurrence cannot be determined by my results.

Models from OFT rely heavily on assumptions and need to be tailored to species and environment (Krebs & Stephens, 1986; Newman et al., 1995). One extensively discussed assumption is that animals wish to optimize the best forage, a "currency" (Pyke et al., 1977; Westoby, 1978), but herbivores can be time-minimizers instead of energy-maximisers (Bergman et al., 2001), and they sometimes feed on low quality forage, even though there is an abundance of high quality forage (Bozinovic & Martínez del Río, 1996). Abundance accessibility of food is also an assumption in OFT (Pyke et al., 1977), but Fryxell (1991) presented a model explaining why low or intermediate forage abundance might be beneficial for herbivores, and van Wieren (1996) suggests that intake rate is more important than forage quality for herbivores. This might also be the case for Svalbard reindeer, as findings from van der Wal (2000) about Svalbard reindeer selecting for quantity instead of quality, directly contradict some of the assumptions that OFT make. Thus, classical OFT might not suit the Svalbard reindeer as well as initially predicted.

4.8 Is faecal C:N ratio an adequate measurement for diet quality?

I chose to use faecal C:N ratio as a "currency" in my study, but the use of faecal N as a proxy for diet quality is discussed (Hobbs, 1987). Faecal N is influenced by microbial abundance in digestive systems which may vary among species (Reese et al., 2018), and one should be careful comparing diet quality among different species, populations and seasons based on faecal N (Hobbs, 1987; Leslie et al., 2008). Faecal N is a result from dietary intake, but handling time and digestion rate also affects nutritional value (Hanley, 1997). Some plants can contain less N, but have faster uptake and passage rate through the digestive system (Van Soest, 1994). The proportion of N in faeces and N-uptake is therefore not necessarily linear. Still, faecal N is regarded an adequate diet quality measurement for herbivores given the right

study design (Leslie et al., 2008; Leslie & Starkey, 1987). My study is limited to one population within a short time frame, and should therefore suit the use of N. The use of C:N ratio N is also debatable, as many studies only use faecal N, but C:N ratio in my study was closely correlated to N content in faeces (Pearson's r = -0.74, figure 2) and should provide similar results. In addition, a recent study by Beumer et al. (2017) used C:N ratio in faeces as a diet quality proxy for Svalbard reindeer, when trying to identify how snow cover properties influenced diet quality during winter.

There have been conflicting findings on what the most constraining compound in an herbivore diet is. Usually energy demands (Owen-Smith & Novellie, 1982) or N (Mattson, 1980; Parker et al., 2009) is suggested, but Belovsky (1978) found that moose (*Alces alces*) was constrained by sodium demands and didn't forage on the most energetic plants because of this. Also, some argue that Phosphorus in addition to N should be measured when investigating diet quality using faeces (Grant et al., 1995). Tannins and defence compounds in plants should also be measured, as this influences digestible N according to Wrench et al. (1997). For Svalbard reindeer it has been suggested that moss could be an important part of diet, due to the high mineral content in moss, but the same authors found that grass and sedges were highly digestible and had high mineral availability (Staaland et al., 1988). In plants the most important nutrients for herbivores, like digestible energy, protein, and phosphorus tend to covary (Van Soest, 1967), and this has been used as an assumption in other studies (Owen-Smith & Novellie, 1982). Therefore, I assume that my use of C:N ratio in this study is an adequate measurement for diet quality.

I also assume that metabarcoding provides adequate measurements of plant family proportions and diversity. DNA methods are sensitive to contamination, but the plant families I found, is in line with many other diet analyses from the Svalbard reindeer (Bjørkvoll et al., 2009; Staaland et al., 1983; Bjune, 2000), thus I expect that metabarcoding provide adequate results on diet composition.

4.9 Sources of error

In my study I have only 47 samples from 25 animals. Low samples size may result in spurious random results, which is how I interpret the unexpected effects of April weight and proportion of grass inside a daily home range on diet quality.

Different aspects than forage quality can influence feeding behaviour for Svalbard reindeer even though the most common disruptive mechanism like predation or insect harassment are absent (Reimers, 1977). Although the general perception is that Svalbard-reindeer is not scared by humans, Svalbard-reindeer show anti-predator behaviour when they avoid snowmobile-tracks during winter (Tandberg, 2016). This could mean that the study-design with replicated sampling of individuals could disrupt their normal feeding pattern, as they ran away when we approached them to pick up a faecal sample. Also, the vegetation map I used to identify habitat within the home ranges may be inaccurate (V. Ravolainen personal communication). It is based satellite images, which mainly were taken around the year 2000 and could have shortcomings, especially on finer scales (Johansen et al., 2012). I believe that the most important shortcoming is the lack of data on fine enough spatial and temporal scale down to the level of selection of individual plant parts.

4.10 Conclusion

Female Svalbard reindeer showed a significant individual difference in diet quality. My study is the first to combine diet data derived from DNA metabarcoding and diet quality as measured by C:N ratio in Svalbard reindeer. This difference in diet quality was not explained by any of the variables I had data on, including diet diversity, plant families in diet, home range size, movement characteristics, habitat selection or individual attributes such as age and body mass. The significant individual variation suggests that there are additional factors causing this that remained undetected in my study. I regard fine scaled foraging behaviour in bite and patch-selection plausible mechanism. The lack of significant results further suggests that overall quality of diet of Svalbard reindeer in summer is high and that many large scale strategies and plant species in the diet yield the same high energy gain.

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Appendix

Appendix 1. Summary of the four primer sets used for the DNA metabarcoding diet analysis of Svalbard reindeer.

Name	Target gene	Target taxon	Sequence (5'-3')	Mean size (bp)	Reference
Euka02	18S rDNA (V7)	Eukaryotes	TTTGTCTGSTTAATTSCG TCACAGACCTGTTATTGC	123	Guardiola et al., 2015
Fung01	ITS1 nuclear rDNA	Fungi	GGAAGTAAAAGTCGTAACAAGG CCAAGAGATCCGTTGYTGAAAGT	226	Epp et al., 2012
Bryo01	P6 loop trnl	Bryophytes	GATTCAGGGAAACTTAGGTTG CCATYGAGTCTCTGCACC	53	Epp et al., 2012
G/H	P6 loop trnl	Plants	GGGCAATCCTGAGCCAA CCATTGAGTCTCTGCACCTATC	48	Taberlet et al., 2007

Appendix 2. Summary of the six synthetic DNA stretches used as PCR positive control mock community with the G/H primers.

Name	Sequence (5'-3')	Size (bp)	GC content (%)
Standard 1	taagtctcgcactagttgtgacctaacgaatagagaattctataagacgtgttgtcccat	60	40
Standard 2	gtgtatggtatatttgaataatattaaatagaatttaatcaatctttacatcgcttaata	60	20
Standard 3	cacaatgctcggtaactagaagcatttgta	30	40
Standard 4	attgaatgaaaagattattcgatatagaat	30	20
Standard 5	agaacgctagaatctaagatggggggggggggatgagtaagatatttatcagtaacatatga	60	40
Standard 6	atttttgtaactcattaacaattttttttttgatgtatcataagtactaaactagttact	60	20