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## Age class, density and temporal effects on diet composition of sheep in an alpine ecosystem

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### Abstract

Understanding diet selection is important since diet determines energy intake and therefore growth of ungulate populations. Yet very few studies have reported annual variation in diet. Density-dependent diet choice by large herbivores has been reported several times, but these studies are typically either short-term or they lack replication of the density treatment. In a landscape-scale experiment with 3 replicates of two densities (25 and 80 individuals/km<sup>2</sup>) of domestic sheep, we determined diet composition using microhistological analysis during 6 summer grazing seasons (2002–2007) in alpine habitats. We tested how age class, density and temporal variation (within season, annually, and over years) affected summer diet. There was marked evidence of additive effects of these factors on overall diet composition, but interactions were few. The interaction between density and annual variation was an important determinant of the proportion of the main forage component (*Avenella flexuosa*), but not of the proportions of herbs, *Salix* spp. and for “other” forage plants. Surprisingly, the density effect on this intermediate quality forage (*A. flexuosa*) was not consistent among years (both positive, negative and no effects), likely arising due to large variation in the proportion of the other forage plants. We discuss how foraging ecology can supplement the insight from life history theory in explaining variation in vital rates.

### Zusammenfassung

Erkenntnisse zur Nahrungswahl sind wichtig, da die Nahrung die Energieaufnahme und damit das Wachstum von Ungulatenpopulationen bestimmt. Allerdings haben nur wenige Untersuchungen über die jährliche Variation der Nahrung berichtet. Dichtabhängige Nahrungswahl ist verschiedentlich bei großen Pflanzenfressern beobachtet worden, aber diese Studien waren typischerweise entweder von kurzer Dauer oder unrepliziert. In einem Experiment auf Landschaftsebene mit drei Wiederholungen von zwei Dichten von Hausschafen (25 und 80 Ind./km<sup>2</sup>), bestimmten wir die Nahrungszusammensetzung mit mikrohistologischen Analysen während sechs Sommerweideperioden (2002–2007) in alpinen Lebensräumen. Wir testeten wie Altersklasse, Besatzdichte und die zeitliche Variation (Probenahmetermin, Jahr und Jahresvergleich) die Sommernahrung beeinflussten. Es gab deutliche Belege für additive Effekte dieser Faktoren auf die Nahrungszusammensetzung, aber nur wenige Interaktionen. Die Interaktion zwischen Besatzdichte und jährlicher Variation war ein wichtiger bestimmender Faktor für

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den Anteil der Hauptfutterkomponente (*Avenella flexuosa*), nicht aber für die Anteile der Kräuter, von *Salix* spp. und der “anderen” Futterpflanzen. Überraschenderweise war der Dichteeffekt auf die Futterpflanze von mittlerer Qualität (*A. flexuosa*) nicht einheitlich über alle Jahre (positiver, negativer und ohne Effekt), was vermutlich auf die große Variation im Anteil der übrigen Futterpflanzen zurückgeht. Wir diskutieren, wie die Ökologie des Nahrungserwerbs Erkenntnisse aus der Theorie der Lebensgeschichte ergänzen kann, um die Variation von demographischen Parametern zu erklären.

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## Introduction

Life history theory and the incorporation of interactions between age, density and climate in vital rates have successfully enabled prediction of population dynamics of large herbivores (Coulson et al. 2001). Some of this variation clearly results from how mothers allocate resources to self maintenance instead of offspring survival (Gaillard & Yoccoz 2003; Martin & Festa-Bianchet 2010). Less attention has been paid to understand foraging behaviour as one potential proximate mechanism behind density and climate generated variations in demography and population dynamics (but see Simard, Côté, Weladji, & Huot 2008 for a notable exception). Diet choice is particularly important with its direct link to energy intake and hence individual growth (White 1983). It may explain some of the large individual variation seen in ungulate populations. However, obtaining data at the scale of herbivore diet choice is considered particularly challenging. Though numerous methods are available such as rumen or microhistological analysis of faeces (Holechek, Vavra, & Pieper 1982), they are often costly to apply. Compared to the vast literature on habitat selection at coarser scales using either very high frequency (VHF) or global positioning system (GPS) collar technology, the knowledge of variation in diet selection at the plant species or group level comes typically from short-term studies. Therefore, very few studies have addressed possible variation in diet due to age, climate, population density and their interactions despite that this is a core theme in population ecology (Gaillard, Festa-Bianchet, Yoccoz, Loison, & Toigo 2000).

Spatial and temporal scaling play a key role in foraging studies (Senft et al. 1987). Empirical studies can be spatially organized at a coarse scale (landscape), an intermediate scale (habitat and patch selection) or a fine scale (diet selection), and temporally from short-term (hours to days) to intermediate (year) or long-term (trends over years) (Bailey et al. 1996). Density-dependent diet choice has been reported for several large herbivore species; grey kangaroo *Macropus giganteus* (Ramp & Coulson 2002), feral donkey *Equus asinus* (Freeland & Choquenot 1990), white-tailed deer *Odocoileus virginianus* (Daigle, Crête, Lesage, Ouellet, & Huot 2004), mule deer *Odocoileus hemionus* (Nicholson, Bowyer, & Kie 2006), and domestic sheep *Ovis aries* (Kausrud, Mysterud, Rekdal, Holand, & Austrheim 2006). Either these studies have no replication of the density treatment (Daigle et al. 2004; Freeland & Choquenot 1990) or they are short-term

(1 year; Kausrud et al. 2006), and they do not consider that the density effect may interact with climatic conditions. Climate-induced annual variation in plant growth and phenology is a common phenomenon in northern ecosystems (Loe et al. 2005), and we therefore expect large annual variation in the diet possibly interacting with the density effect.

In this landscape-scale experiment, we analysed diet of domestic sheep (ewes and lambs) kept at two densities in alpine habitats during 6 consecutive grazing seasons (2002–2007). In this experiment, body growth of lambs was markedly density-dependent, and the strength of the density effect varied annually (Mørbæk, Mysterud, Holand, & Austrheim in press). We tested the following predictions (P<sub>1</sub>–P<sub>4</sub>) regarding how age class, climate and densities, additively or interactively, affect summer diet:

### P<sub>1</sub>. Diet breadth and composition

Due to the intraspecific competition for forage and observed lower body growth rate at high density, we predicted that sheep at high density would have a broader diet composition with less high-quality forage (herbs) and more low-quality forage (graminoid and woody species) than sheep at low density (McLoughlin, Morris, Fortin, Vander Wal, & Contasti 2010; Morris, 2003).

### P<sub>2</sub>. Seasonal variation

As high-quality herbs are the forage most preferred by sheep (Bowns & Bagley 1986) and due to the gradual loss of high-quality plant material in the course of the year (Mysterud et al. 2011), we predicted herbs to be replaced by graminoids towards the end of the grazing seasons (P<sub>2a</sub>). We further expected that the density-dependent diet choice would be stronger towards the end of grazing season, expressed by a lower intake of herbs and higher intake of graminoids in late season by sheep at high density when compared to low density (date × density interaction) (P<sub>2b</sub>).

### P<sub>3</sub>. Annual variation

Since among-year variation in climate affects primary production in alpine habitats (Mårell, Hofgaard, & Danell 2006), we expected sheep diet to vary among years (P<sub>3a</sub>). We predicted a stronger density dependence in years with poor

vegetation development due to an expected stronger competition for forage; we expected that sheep at high density would consume less herbs and more graminoids than sheep at low density (year  $\times$  density interaction) ( $P_{3b}$ ) in some years with stronger resource limitation. We also tested for trends over years.

#### P<sub>4</sub>. Age class

As nutritional requirements vary as a function of metabolic body mass in ruminants, we predicted that lambs, with their smaller body size and hence higher nutritional requirements (Demment & Van Soest 1985), would select a diet of higher quality, *i.e.* including more herbs and less graminoids than ewes. We also explore interactions between the above factors.

## Materials and methods

### Study area

The study was conducted in Hol municipality, Buskerud county in southern Norway (60°40'N, 7°55'E). The terrain is alpine (1050–1320 m a.s.l.) with dwarf shrub heath as the dominating vegetation type, covering 51% of the enclosure. In terms of biomass, woody species, such as *Betula nana*, *Empetrum nigrum* and *Vaccinium myrtillus* constitute 85% of this plant community, while graminoids add up to 6% and bryophytes and lichens to 8%. The amount of herbs is generally low (1%). Tall herb meadow and low herb meadow, the vegetation types with the highest grazing values for sheep (Mobæk, Mysterud, Loe, Holand, & Austrheim 2009) comprise 9% of the total vegetation cover. Graminoids are abundant in the meadow vegetation (47% of the biomass), with *Carex bigelowii*, *Avenella flexuosa* and *Deschampsia caespitosa* as the most common species. Woody bushes like *Salix lapponum* and *Salix glauca* make up 31% of the biomass in meadow vegetation, while herb species (12% of the biomass) bryophytes and lichens (9% of the biomass) are less abundant in meadow vegetation. In general, vascular plant richness is high in the study area, with herbs constituting 53% of all vascular plant species (Austrheim, Evju, & Mysterud 2005). Before fencing off the experimental enclosure (2001), there was a relatively low grazing pressure by domestic sheep in the area (<10 sheep/km<sup>2</sup>), and only sporadic visits by moose (*Alces alces*) and individual or small herds of wild reindeer (*Rangifer tarandus*).

### Experimental design

The large experimental enclosure (2.7 km<sup>2</sup>) was split into nine sub-enclosures to allow a block-wise randomized design with 3 replicates (Austrheim et al. 2008). The sub-enclosures averaged 0.3 km<sup>2</sup> in size. The actual sizes ranged from 0.22 to 0.38 km<sup>2</sup>, which was caused by practical problems when

putting up fences in the rocky area. Sub-enclosures were designed to ensure that each of them enclosed roughly the same altitudinal span and the same distribution of main vegetation types used by sheep. For each of three neighbouring sub-enclosures, one was randomly selected as control (no sheep), one as low-density and one as high-density sub-enclosure. A botanical examination and estimation of the vegetation types' grazing value for sheep was conducted in 2001, and based on recommendations provided here, 25 sheep/km<sup>2</sup> and 80 sheep/km<sup>2</sup> were chosen as low and high densities, respectively. These sheep density levels are typical for alpine summer pastures in southern Norway, and correspond to low and moderate grazing pressure as measured by grazing frequency of the most important forage plants in our study area (Evju, Mysterud, Austrheim, & Økland 2006).

Each grazing season from 2002 onwards, 24 or 25 lactating ewes and 44 or 45 lambs belonging to the same sheep farmer were released into the enclosure. The actual number of sheep in treatments ranged from 2–3 ewes with lambs (low density) to 5–6 ewes with lambs (high density). Ewes with one, two or three lambs were distributed evenly between the high and low density treatment sub-enclosures. To allow individual recognition, both ewes and lambs were marked with colour-coded neck bands in addition to the individually numbered plastic ear tags. All sheep were of the breed “Norsk Kvit Sau” (also known as the “Dala” breed), which is a heavy and the most common breed kept by Norwegian sheep farmers. Ewes weighed on average 81 kg when they were released into the sub-enclosures, and lambs weighed on average 20 kg when released and 42 kg when recaptured in autumn. A grazing season typically lasted from late June to late August (see Appendix A, Table 1), which is the normal length of the summer grazing season for sheep on alpine pastures in southern Norway.

### Data collection

Individual sheep were followed during the entire summer grazing seasons from 2002 to 2007. Direct observations of defecating ewes and lambs were obtained from a distance of 20 to 50 m using binoculars (see Mobæk et al. 2009 for details on sampling times). Faeces samples were collected from known individuals only, and a careful sampling procedure ensured near equal representation of densities (high *vs.* low), age class (ewe *vs.* lamb) (see Appendix A, Table 1) and 3 time periods (early, middle and late grazing season). All faeces samples were put in plastic bags and frozen, and later a stratified assortment (consisting of 441 samples from 236 individual sheep) of the total sampled material was selected for analyses of diet. Microhistological analyses (Holeček et al. 1982) were performed following a standard procedure (boiling 1 ml of faeces in 4 mm of nitric acid). Each faeces sample was split into two parallel sub-samples that were processed independently. The mean number of faeces samples analyzed per individual sheep was 1.88 ( $\pm 1.35$  SD). Plant

fragments were identified and counted at the level of species whenever possible, otherwise family names were determined. Most herb species (except *Geranium silvaticum*, *Viola biflora* and *Liliaceae*, all constituting a minor component of diet) were particularly difficult to identify and hence they were only recorded at plant group level. Unidentified fragments were not considered and do not form part of the percentage calculations.

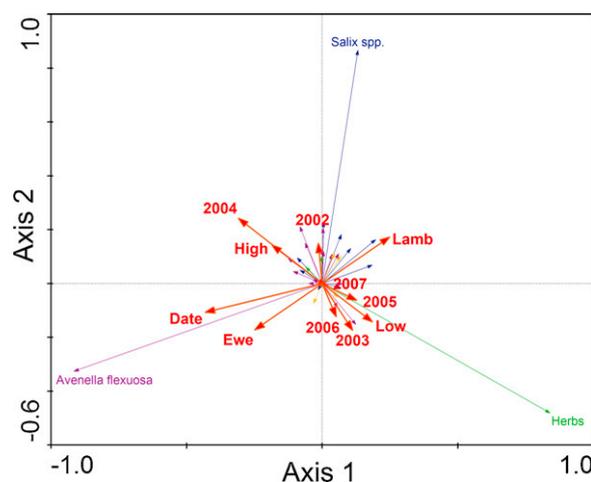
## Statistical analyses

### Multivariate gradient analyses

Diet composition was estimated as proportions (%) of the individual plant taxa within a particular faeces sample. Proportions were arcsine[ $\sqrt{\text{proportion}/100}$ ]-transformed to avoid heteroscedasticity. We used ordination methods to investigate how sheep's diet (selection of the different plant taxa) varied as a function of sheep density, age class, date (Julian date) and year. Ordination analyses are particularly useful when large numbers of species are involved (Ter Braak & Smilauer 2002). In this case, a total of 33 plant taxa were identified in the faeces samples and employed as response variables. To choose which ordination methods to apply, the total beta diversity or "gradient length" (a measure of change in species along environmental gradients) was calculated using a detrended correspondence analysis (DCA), which is often used to cope with arch effects commonly produced by correspondence analysis (CA) (Leps & Smilauer 2003). Unimodal ordination methods are appropriate when the length of the first DCA ordination axis is  $\geq 4$  SD (SD; unit of species turnover on ordination axis), whereas a linear ordination method should be applied if the gradient length is  $\leq 2$  SD (Ter Braak & Smilauer 2002). For intermediate lengths both methods would be useful, however Ter Braak and Prentice (1988) advise unimodal models when the gradient length exceeds 3 SD and linear models below this value. A DCA showed that the gradient length for our species data/response variables was 2.062 SD, suggesting that our species responses might be close to linear (Jongman, Braak, & van Tongeren 1995). Consequently we proceeded with principal component analysis (PCA) and redundancy analysis (RDA), both ordination methods assuming a linear response. In RDA the axes are constrained to be linear combinations of the explanatory variables (*i.e.* a constrained PCA), a method that is suitable when gradients are short ( $\approx 2$  SD) (Ter Braak & Smilauer 2002). We entered each of the explanatory variables in the RDA model by manual forward selection and tested them for significance using Monte Carlo permutations (number of unrestricted permutations = 499). DCA, PCA and RDA ordination analyses were performed with Canoco for Windows 4.5.

### Generalized linear mixed-effects models

Based on the PCA of the total diet composition, the most dominant plant taxa in sheep's diet, *A. flexuosa*, herbs, *Salix*



**Fig. 1.** Species–environment biplot from a PCA of plant species composition in sheep diets ( $n=441$  faeces samples) in relation to sheep density (high or low), age class (ewe or lamb), date (Julian date) and grazing season (2002–2007). Eigenvalues of PCA axes 1 (horizontally) and 2 (vertically) are 0.493 and 0.222, while fractions of variance explained are 0.493 and 0.222, respectively. The axes in this biplot explain 71.55% of the total variance in diet composition, and are not constrained by the explanatory variables (*i.e.* they are passive variables). To ease interpretation of the diagram, all plant species are classified into four different plant groups which are illustrated by colours: blue arrows and species names = woody species, lilac = graminoids, green = herbs and yellow = cryptogams. See Appendix A, Table 3 for details.

spp. (see Fig. 1), and "other forage plants" were selected for more detailed statistical analyses with a focus on determining interactions. First, we used plotting with general additive models (GAMs) to search for possible non-linearity in our data. Then, we fitted generalized linear mixed-effects models (GLMM's in library "lme4" in R) to determine how selection of each of these four plant taxa varied with selected interactions between the main factors (see 'Introduction' section).

Mixed-effects models are useful when temporal and spatial pseudoreplication are involved, which is the case for our data with repeated samplings and an experimental set-up with 3 replicates of each density treatment. Thus, we fitted "individual" and "sub-enclosure" as random variables. We used model selection with Akaike Information Criterion (AIC) to find the most parsimonious model. The model with the lowest AIC value (and highest AIC weight) was retained to estimate the parameters for testing our predictions. All modelling was done in R. To estimate confidence limits for the model parameters we constructed highest probability density (HPD) intervals by using Markov chain Monte Carlo simulations in the R library "coda", which is recommended over normal confidence limits for GLMMs fitted with lmer (Pinheiro & Bates 2000).

**Table 1.** *t*-Values of regression coefficients when using the explanatory variables as passive variables in PCA of plant species composition in sheep diet (number of samples = 441). *t*-Values > 2.1 indicate that the variable is important for the species data.

Variable	Axis 1	Axis 2
Date	-14.2469	-5.0988
Age class	-9.9220	-5.0995
Year 2004	-7.0913	4.4067
Density	-4.6049	5.0685
Year 2002	-1.4672	2.6894
Year 2005	1.4207	-1.2278
Year 2003	0.8087	-3.7259
Year 2006	-0.0615	-2.0870
Year 2007	-	-

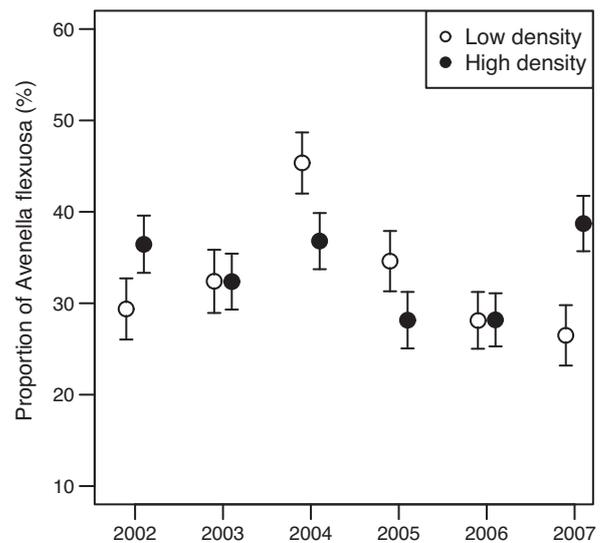
**Table 2.** Results from RDA using the explanatory variables in a constrained ordination of plant species composition in sheep diet (number of samples = 441). All explanatory variables are tested for significance by Monte Carlo permutations (number of unrestricted permutations = 499).

Explanatory variable	Variance explained	<i>F</i> -ratio	<i>P</i> -value
Date	0.10	85.22	0.002
Year 2004	0.06	54.48	0.002
Age class	0.05	53.88	0.002
Year 2002	0.02	23.14	0.002
Density	0.02	19.86	0.002
Year 2003	0.02	15.49	0.002
Year 2005	0.01	9.99	0.002
Year 2006	0.00	5.45	0.004
Year 2007	-	-	-

## Results

A PCA of the total diet composition indicated that the sheep's diet varied within a season and among years, and that both sheep age class and density affected the quantity of different plant taxa in the diet (Fig. 1). Both PCA (Table 1) and RDA (Table 2) ranked date as the most important factor determining a sheep's diet, followed by year, age class and density, respectively. The most frequently eaten plants by sheep (given as a mean for samples in all years) were the grass *A. flexuosa* (31.4 ± 14.5% SD), herbs (24.3 ± 13.5% SD) and *Salix* spp. (12.3 ± 9.8% SD). We pooled "other plants" in further analyses focussing on potential interactions, though they do not necessarily form a coherent functional group.

In the multivariate analysis, we found a strong positive correlation between the proportion of herbs in the diet and low sheep density, and positive correlations between *A. flexuosa* and *Salix* spp. and high sheep density (Fig. 1). This supported our prediction regarding a lower quality diet at high density (P<sub>1</sub>). The interaction between year and density entered the best mixed model only for *A. flexuosa* (see Appendix A,



**Fig. 2.** Proportion of *Avenella flexuosa* in diets of sheep at low and high densities for the summer grazing seasons 2002–2007 in Hol, Norway, based on the best mixed model in Appendix A, Table 2 (for levels "ewe" and Julian date = 200). Error bars represent standard errors.

Table 2). There was thus only some support for our prediction that the effect of density depends on year (P<sub>3b</sub>). For *A. flexuosa*, sheep at high density consumed both more and less of this grass than sheep at low density in a specific year with density-dependent diet choice (Table 3 and Fig. 2). Overall diet varied between years, in accordance with P<sub>3a</sub> (Fig. 1). The sheep's intake of herbs, *Salix* spp. and "other plants" showed marked annual variation also in the mixed models (see Appendix A, Tables 2 and 3). Intake of herbs and *Salix* spp. decreased and "other plants" increased at high compared to low density, but the confidence intervals marginally overlapped zero (Table 3). Thus, P<sub>1</sub> was only partly supported in the mixed models.

The interaction between density and date was not included in any of the best models for either *A. flexuosa*, herbs, *Salix* spp. or "other plants" (see Appendix A, Table 2), so we found no support for our prediction P<sub>2b</sub> that density dependent selection was stronger in the late grazing season. As predicted in P<sub>2a</sub>, sheep's intake of high-quality herbs and *Salix* spp. declined towards the end of the grazing seasons and was replaced by a higher intake of *A. flexuosa* (Table 3). The selection of "other plants" remained constant throughout the season (Table 3).

The age class effect did not interact with either density, seasonal, annual variation or the trend over years (see Appendix A, Table 2). Age class affected sheep's diet composition with lambs eating more high-quality plant material than ewes, which is in accordance with P<sub>4</sub>. The mixed models also confirmed the pattern found in the PCA analysis, with lambs consuming less *A. flexuosa* and "other plants", but more herbs and *Salix* spp. than ewes (Fig. 1 and Table 3).

**Table 3.** Generalized linear mixed effects model for the proportion of *A. flexuosa*, herbs, *Salix* spp. and “other plants” in the diet of sheep at low and high density treatment for the summer grazing seasons 2002–2007 in Hol, Norway. The parameter estimates are based on the best models (see Appendix A, Table 2 The 95% HPD intervals for fixed effects not including 0 are bolded (number of samples = 784, sheep ID = 236, sub-enclosure = 6).

Fixed effects	<i>A. flexuosa</i>			Herbs			<i>Salix</i> spp.			Other plants		
	Estimate	Lower 95%	Upper 95%	Estimate	Lower 95%	Upper 95%	Estimate	Lower 95%	Upper 95%	Estimate	Lower 95%	Upper 95%
Intercept	-0.054	-0.168	0.064	<b>0.9605</b>	<b>0.8499</b>	<b>1.0697</b>	<b>0.6336</b>	<b>0.5173</b>	<b>0.7447</b>	<b>0.6717</b>	<b>0.5713</b>	<b>0.7698</b>
Density (low vs. high)	<b>-0.101</b>	<b>-0.192</b>	<b>-0.018</b>	0.0580	-0.0173	0.1305	0.0452	-0.0372	0.1365	-0.0538	-0.1225	0.0105
Date	<b>0.004</b>	<b>0.003</b>	<b>0.004</b>	<b>-0.0026</b>	<b>-0.0030</b>	<b>-0.0021</b>	<b>-0.0015</b>	<b>-0.0020</b>	<b>-0.0011</b>	-0.0003	-0.0006	0.0002
Year (2003 vs. 2002)	<b>-0.057</b>	<b>-0.102</b>	<b>-0.009</b>	<b>0.0989</b>	<b>0.0633</b>	<b>0.1318</b>	<b>-0.0543</b>	<b>-0.0881</b>	<b>-0.0168</b>	<b>-0.0566</b>	<b>-0.0874</b>	<b>-0.0247</b>
Year (2004 vs. 2002)	-0.002	-0.050	0.042	<b>-0.1121</b>	<b>-0.1473</b>	<b>-0.0796</b>	0.0345	-0.0012	0.0678	-0.0022	-0.0337	0.0289
Year (2005 vs. 2002)	<b>-0.091</b>	<b>-0.136</b>	<b>-0.044</b>	<b>0.0604</b>	<b>0.0267</b>	<b>0.0934</b>	-0.0212	-0.0542	0.0129	-0.0089	-0.0384	0.0218
Year (2006 vs. 2002)	<b>-0.089</b>	<b>-0.133</b>	<b>-0.044</b>	0.0175	-0.0152	0.0514	<b>-0.1127</b>	<b>-0.1459</b>	<b>-0.0790</b>	<b>0.0899</b>	<b>0.0588</b>	<b>0.1205</b>
Year (2007 vs. 2002)	0.003	-0.048	0.051	-0.0012	-0.0361	0.0351	<b>-0.0507</b>	<b>-0.0859</b>	<b>-0.0137</b>	<b>0.0401</b>	<b>0.0070</b>	<b>0.0725</b>
Age cl. (lamb vs. ewe)	<b>-0.090</b>	<b>-0.112</b>	<b>-0.070</b>	<b>0.0647</b>	<b>0.0435</b>	<b>0.0859</b>	<b>0.0746</b>	<b>0.0524</b>	<b>0.0961</b>	<b>-0.0223</b>	<b>-0.0417</b>	<b>-0.0027</b>
Year (2003 vs. 2002) × density (low vs. high)	<b>0.126</b>	<b>0.054</b>	<b>0.197</b>									
Year (2004 vs. 2002) × density (low vs. high)	<b>0.176</b>	<b>0.105</b>	<b>0.244</b>									
Year (2005 vs. 2002) × density (low vs. high)	<b>0.162</b>	<b>0.094</b>	<b>0.230</b>									
Year (2006 vs. 2002) × density (low vs. high)	<b>0.107</b>	<b>0.039</b>	<b>0.173</b>									
Year (2007 vs. 2002) × density (low vs. high)	-0.034	-0.110	0.036									
Random effects	<i>A. flexuosa</i>			Herbs			<i>Salix</i> spp.			Other plants		
	SD	Lower 95%	Upper 95%	SD	Lower 95%	Upper 95%	SD	Lower 95%	Upper 95%	SD	Lower 95%	Upper 95%
Sheep ID	0.098	0.043	0.059	0.107	0.048	0.063	0.116	0.047	0.063	0.103	0.042	0.056
Sub-enclosure	0.050	0.021	0.094	0.051	0.022	0.090	0.046	0.015	0.092	0.057	0.019	0.085

## Discussion

Evidence for density dependence (reviews in Bonenfant et al. 2009; Fowler 1987) and climate-induced annual variation in demographic rates are persuasive (reviews in Gaillard et al. 2000; Mysterud, Stenseth, Yoccoz, Ottersen, & Langvatn 2003; Weladji, Klein, Holand, & Mysterud 2002). The mechanisms behind these patterns are to a large extent expected to operate through diet quality or quantity (Simard et al. 2008). The results from this first fully replicated experimental, long-term study of sheep's diet highlight how the combination of age class, density and temporal variation determine diet composition (Fig. 1). There was marked evidence that age class, density and temporal variation additively affected diet, but less strong evidence of interactions, apart from the density and year interaction in *A. flexuosa* (Fig. 2). The strong annual variation that interacts with the density effect might be expected to give rise to similar variation in vital rates, and indeed, annual and density-dependent body growth of lambs interacted (Møbak et al. in press).

From the demographic and life history viewpoint, the annual scale and density dependence are arguably the most interesting. In the ordination of the overall diet composition, there was evidence for both density dependence and annual variation (Fig. 1 and Tables 1 and 2). The strong annual variation in diet suggests changes in species specific biomass or quality. We have earlier reported evidence for marked variation in herb growth among years (Møbak et al. 2009), which likely have consequences for herbivore body mass gain during summer. Reduced growth by lambs at high density (Møbak et al. in press) is likely to arise partly due to differences in diet composition at the two density levels. We expected less herbs to be eaten in years with little forage biomass, and similarly, high density of sheep to increase competition for forage reducing intake of high quality forage. Further, we predicted inter-annual climatic variation to interact with the density effect such that a stronger density-dependent selection would occur in years with poor vegetation development. The interaction term between annual variation and density entered the best model for *A. flexuosa* only (see Appendix A, Table 2 and Fig. 2). For herbs, assumed to be of high quality (Bowns & Bagley 1986), there was marked annual variation and a tendency towards a lower proportion eaten at high compared to low density. A similar pattern occurred for *Salix* spp. Among the "other forage plants", which do not form a consistent functional group, many species can be assumed to be of fairly low nutritional value according to the low intake rates. For this group, there was a tendency towards an increase in the proportion at high density. For the intermediate quality forage, the graminoid *A. flexuosa*, the effect of density between years therefore was not consistent, as it depended on the combined responses of the high quality herbs, *Salix* spp. and the low quality "other plants". Therefore, intermediate quality forage may show opposite density dependence between years.

Climatic conditions influence plant phenology (Mårell et al. 2006), and thereby affect habitat selection and in turn herbivore performance (Pettorelli, Mysterud, Yoccoz, Langvatn, & Stenseth 2005). In alpine ecosystems, high spring/summer temperatures and little snow typically benefit rapid plant growth and early maturation, while cold weather reduces plant growth and also slows down snow melt prolonging access to newly emerged high-quality forage (Hebblewhite, Merrill, & McDermid 2008). There was clear evidence that seasonal progression in plant development was important for diet composition. The intake of herbs and *Salix* spp. gradually declined and was replaced by grasses such as *A. flexuosa* towards the end of the grazing seasons. Although we expected the density-dependent selection to be stronger in the late grazing season when both forage availability and plant quality was reduced, we found no strong interaction between date and density for any of our focal forage plants, suggesting this was a short-term effect found only in the first year of our study (2002; Kausrud et al. 2006). At longer time scales, intense selective feeding may affect the availability of important forage species with repercussion on life history (Simard et al. 2008). However, such effects are fairly moderate within the time frame of our study (Austrheim et al. 2008; Evju, Austrheim, Halvorsen, & Mysterud 2009), and this may explain no clear trends in diet over years (see Appendix A, Table 2). In large herbivores, juveniles are more sensitive to both environmental fluctuations and density dependence than adults (Gaillard, Festa-Bianchet, & Yoccoz 1998). During periods with resource shortage, ungulate females may favour their own mass gain rather than the growth of their offspring (Gaillard & Yoccoz 2003; Martin & Festa-Bianchet 2010). For example, lactating bighorn sheep (*Ovis canadensis*) provided less maternal care and produced lighter lambs in years with high population density and low resource availability than in years with low population density and, hence, less food competition (Festa-Bianchet & Jorgenson 1998; Martin & Festa-Bianchet 2010). In accordance with the energy-body size allometric theory (Demment & Van Soest 1985), we found that lambs selected more high-quality herbs and less grass than ewes. Our data supported only a main effect of age class, and we failed to find any interactions between age class and density or temporal variation on diet.

Our study quantifying age class, density and temporal effects on the diet of a large herbivore increase our understanding of mechanisms determining vital rates. We document density dependence and annual variation in diet, which may form one potential mechanism of similar patterns found in lamb body growth. Reduced body growth during the first year of life typically delays age at first reproduction by one year (Langvatn, Mysterud, Stenseth, & Yoccoz 2004). Our results for *A. flexuosa* suggest that interactions can arise due to diet composition effects. We suggest that more studies on proximate mechanisms can further advance our understanding of factors limiting ungulate populations.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2012.06.009>.

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