



Short Note

Quantification of sex-related diet composition by free-ranging mountain hares (*Lepus timidus*)

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Abstract

Variability in habitat use influences animal distribution and can lead to different life-histories across sexes and individuals. Such differences are frequently observed in species with sexual dimorphism, but less known in monomorphic species. In this study, we investigated sex-related differences in the diet of the mountain hare (*Lepus timidus*) — a monomorphic species — during the mating season (spring) and the post-reproductive period (autumn). We investigated the diet composition by the microhistological analysis of pellets of 18 individuals (8 males, 10 females) which were surveyed from 2015 to 2017 by a non-invasive genetic population monitoring in the Swiss National Park. We found significant differences in diet composition between seasons and sexes. Females consumed a higher proportion of graminoids in the mating season, probably due to higher energetic needs at that time of the year. Our study shows that further attention should be given to sex-specific diet composition of monomorphic species.

Sexual segregation in the use of resources (incl. habitat and forage) influences animal distribution and survival (Ruckstuhl and Neuhaus, 2004; Isaac, 2005) and can lead to different life-histories across sexes (Ostfeld, 1990; Wauters and Dhondt, 1992; Hoffman et al., 2008). For example, males and females often allocate resources differently during the mating season and the reproductive season (Clutton-Brock, 1989; Hoffman et al., 2008). Sexual segregation is frequently observed in species with sexual dimorphism, i.e., species in which males are clearly larger than females, such as ungulates (Main, 1988; Ruckstuhl and Neuhaus, 2004). However, it is less studied in monomorphic species (Phillips et al., 2011).

Male and female mountain hares (*Lepus timidus*) have similar body sizes with an average weight between 2.2 and 2.8 kg in the Alps (Rehnus, 2013). Thus, the species is a suitable model for analyzing the knowledge gap in sex-related diet composition. During the mating season, females have been shown to have higher glucocorticoid metabolite levels than males, which can lead to higher energetic demands (Rehnus and Palme, 2017). This difference disappears in the post-reproductive period (Rehnus and Palme, 2017). The social hierarchy of hares, which includes dominant and subdominant individuals, may also influence diet composition (Hewson, 1990). We used data from a recently established non-invasive genetic monitoring study of free-ranging mountain hares (Rehnus and Bollmann, 2016) to test whether sexual segregation in diet can be found during the mating season but not during the post-reproductive period. Because females have higher energy requirements during the reproduction period (Kenagy et al., 1989), we expect to see differences between sexes.

Our study area comprises 3.5 km² and is situated along the Ofenpass in the Swiss National Park in southeastern Switzerland (46°39' N, 10°11' E). The Swiss National Park is designated by the International

Union for the Conservation of Nature (IUCN, 2016) as a Category Ia nature reserve (strict nature reserve/wilderness area). It is closed to the public during sampling in spring; during sampling in autumn, it is open but hiking is limited to walking routes and grazing by domestic animal is prohibited. The study area was selected to represent the ecological range occupied by the mountain hare in the Swiss National Park and encompasses seven main habitat types: meadows (29%; with diverse grasses and sedges), timber stands (24%), scree slopes (16%), storeyed stands (12%; mixed *Larix decidua*, *Pinus cembra*, *P. sylvestris*, *P. mugo* spp., *Picea abies*), sapling stands (6%; dominated by *P. mugo* spp.), pole timber (5%), and mature stands (5%). Residual habitats cover 3% of the area. The climate in the Swiss National Park is continental, with mean January and July temperatures of -9 °C and 11 °C, respectively (Haller et al., 2013). The monthly mean precipitation measured at 1970 m a.s.l. is 34 mm in January and 108 mm in July (Haller et al., 2013). The area is accessible under different snow conditions with a minimal risk of avalanches. The genetic monitoring of the population allows us to distinguish samples between mountain hare and European hare (*Lepus europaeus*) which has been shown to rarely occur in high altitudes in the Alps (Bisi et al., 2015).

We collected fresh pellets during three mating seasons (end of March until first half of April) and three post-reproductive periods (October)

Table 1 – Number of samples (unique individual) found during sampling in three mating seasons (end of March until first half of April) and three post-reproductive periods (October) from 2015 to 2017 in the Swiss National Park.

Sex	2015		2016		2017	
	Mating season	Post-reprod. period	Mating season	Post-reprod. period	Mating season	Post-reprod. period
Male	5(4)	0(0)	3(3)	1(1)	2(1)	1(1)
Female	3(3)	2(2)	3(3)	7(5)	4(3)	5(3)

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Table 2 – Diet composition by mountain hares in the mating season (end of March until first half of April) and in the post-reproductive period (October) from 2015 to 2017 in the Swiss National Park.

Season	Sex	Graminoids [Mean ± SD]	Forbs [Mean ± SD]	Dwarf-Shrubs [Mean ± SD]	Coniferous trees [Mean ± SD]	Mosses [Mean ± SD]
Mating season	all	8.6 ± 7.8	0.3 ± 0.5	7.5 ± 6.9	75.6 ± 16.3	0.0 ± 0.1
	Male	5.1 ± 7.1	0.2 ± 0.4	6.9 ± 2.6	80.5 ± 9.1	0.0 ± 0.1
	Female	11.5 ± 7.4	0.5 ± 0.5	8.1 ± 9.4	71.3 ± 20.3	0.0 ± 0.1
	<i>p</i> -value	0.048	0.180	0.876	0.254	0.942*
Post- reproductive period	all	68.6 ± 19.7	3.2 ± 2.7	4.7 ± 4.7	17.4 ± 18.9	0.2 ± 0.3
	Male	70.4 ± 6.8	3.0 ± 4.2	4.3 ± 3.8	15.8 ± 19.1	0.8 ± 0.2
	Female	68.2 ± 21.8	3.3 ± 2.6	4.8 ± 5.1	17.8 ± 20.0	0.0 ± 0.0
	<i>p</i> -value	0.826	0.778	0.976	0.914	<0.001*
Inter- seasonal differences	<i>p</i> -value	<0.001	<0.001	0.227	<0.001	0.107*

* low sample sizes.

from 2015 to 2017 (Tab. 1). Samples were collected both systematically and opportunistically, as described in detail by Rehnus and Bollmann (2016). DNA samples were amplified in three independent replicates and genotypes were estimated according to Rehnus and Bollmann (2016) and Schenker et al. (2020).

Dietary composition was assessed by analyzing two pellets per sample with microhistological methods following a standardized procedure (Suter et al., 2004) established for mountain hares (Rehnus et al., 2013). Briefly, fragments were classified in broad taxonomical groups according to key epidermal characteristics and frequency of occurrence (Chapuis, 1990). The fragments were then aggregated to the five main botanical categories of the study area: graminoids (e.g. *Nardus stricta*, *Festuca* sp., *Poa* sp., *Agrostis* sp., *Luzula* sp., *Carex* sp.), forbs, dwarf shrubs (e.g. *Erica carnea*, *Vaccinium myrtillus*, *Rhododendron ferrugineum*), coniferous trees (e.g. *Picea abies*, *Pinus* sp.), and moss species (Rehnus et al., 2013). When identification was impossible, epidermal fragments were classified as unidentified.

To estimate sex-related differences in diet composition, we used the identified epidermal fragments and compared the proportion of each botanical group per sample for each unique individual per season (N=28) across sexes. Prior to, we tested the influence of sampling year, habitat type at pellet location and season on diet composition by linear models with botanical group as the response variable. Based on the seasonal differences in diet, we tested the differences between sexes for each botanical group for the mating season (N=17) and the post-reproductive period (N=11) using linear models, with botanical group as the response variable and sex as the predictor variable. All statistical tests were conducted using R 3.1.2 (R Development Core Team, 2018). We used Shapiro-Wilk normality test to examine the distribution of data. If variables were not normally distributed, they were transformed to meet the criteria of normal distribution.

Of the 5600 fragments in 28 samples (200 per sample), 5200 fragments could be allocated to a specific botanical group (92.8% of the total), while 400 fragments (7.2%) could not be identified.

We found no influence of habitat type (sites in forests: N=21, in meadows: N=5, in scree: N=2) or year of sampling on diet composition. However, we found seasonal differences for the ratio of coniferous trees ($F_{(1,23)}=68.41$, $r^2=0.72$, $p<0.001$) which had higher ratios during the mating season than during the post-reproductive period (Tab. 2). Graminoids ($F_{(1,23)}=98.92$, $r^2=0.78$, $p<0.001$) and forbs ($F_{(1,23)}=22.94$, $r^2=0.46$, $p<0.001$) were more frequently recorded in the post-reproductive period than during the mating season (Tab. 2). The observed differences in diet between the mating and post-reproductive seasons are in line with findings from other studies, which indicate that mountain hares are generalist herbivores capable of adapting their diets to the seasonal availability (Johannessen and Samset, 1994; Tangney et al., 1995; Loidl, 1997; Hiltunen, 2003; Rao et al., 2003). For example, nearly three-quarters of the mountain hare diet is comprised of coniferous trees during the mating season at a time when most other food resources are covered by snow (Rehnus et al., 2013). In contrast,

diets are largely dominated by more nutrient-rich graminoids during the post-reproductive period (Loidl, 1997; Rehnus et al., 2013), when the study area is free of snow. This seasonal diet composition of the mountain hares can be explained by its intermediate feeding strategy which varies from grazing to browsing on a seasonal basis (Hulbert et al., 2001) and relates to the seasonal change in food availability in the study area which is mainly influenced by snow (Rehnus et al., 2013).

Within seasons, we found that females forage more graminoids than males during the mating season ($F_{(1,15)}=4.66$, $r^2=0.19$, $p=0.048$; Tab. 2). However, this result seems to be biased by the small sample size with two males only. The higher proportion of graminoids for females is likely related to the higher energetic costs of reproduction, namely the activation of catabolic processes associated with the hypothalamic ± pituitary ± adrenal axis activation, ovulation, and pregnancy (Saltzman et al., 1998; Cavigelli et al., 2003; Gittleman and Thompson, 2015; Rehnus and Palme, 2017). Protein content and digestibility of graminoids is highest in the early phase of growth (Albon and Langvatn, 1992; Duru, 1997; Bumb et al., 2016). This sexual difference in nutrient requirements in the late gestation and early reproduction period is in line with observation in other herbivores, e.g. ungulates (Main, 1988; Ruckstuhl and Neuhaus, 2004) and influences diet composition (Bowler, 2004).

Our results indicate that diet composition of mountain hares varies between sexes, despite the lack of a significant sexual dimorphism. However, it should be recognized that also other factors such as the study area or the quantity of forage might affect diet composition. ☞

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