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Seasonal variation in food habits of the Italian hare in a south Apennine semi-natural landscape

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The Italian hare is a species of hare endemic to central and southern Italy and to Sicily. It has been classified as a 'vulnerable' species by the International Union for Conservation of Nature (IUCN), as it is considered to have a high risk of extinction in the next decade. Despite its endangered status, little is known about its feeding habits. In the present study, the seasonal pattern of diet composition of a population of Italian hare occupying a semi-natural landscape was estimated by using the micro-histological technique of faecal analysis. The results showed that hares had a diversified diet, consuming plant parts from over 70 species. Like other *Lepus* sp., the Italian hare consumed a large amount of herbaceous plants (e.g. *Brachypodium sylvaticum*, *Trifolium pratense*, *Allium subhirsutum* and *Festuca arundinacea*), although it complemented its diet seasonally with fruits of *Prunus spinosa*, *Pyrus piraster* and *Malus sylvestris*. Analysis of similarities (ANOSIM) evidenced significant differences among seasons, as a consequence of the seasonal occurrence of the various food items. Spring and autumn ($R = 0.7482$, $P = 0.001$), as well as spring and winter ($R = 0.7398$, $P = 0.001$), showed low diet similarities; these results were supported by similarity percentage analysis (SIMPER, average dissimilarity: > 71% between spring and autumn; > 69% between spring and winter) with taxa like *P. spinosa*, *Cirsium strictus*, *T. pratense* and *Rosa canina* making the greatest contributions to these differences. Higher similarities were instead found when comparing other seasons. This seasonal pattern of diet composition was clearly depicted in the graph from nonmetric multidimensional scaling (n-MDS) ordination. Our results highlight the importance of some plant taxa in the diet of the Italian hare and could be useful in managing reintroduction programs.

KEY WORDS: diet, faecal analysis, *Lepus corsicanus*, micro-histological techniques, multivariate analysis.

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INTRODUCTION

Populations of the Italian hare (*Lepus corsicanus*) have dramatically declined in central and southern Italy during the past few decades, due to illegal hunting, habitat fragmentation and a possible competition with the European hare (*Lepus europaeus*) (Angelici et al. 2008). Consequently, the International Union for Conservation of Nature (IUCN)'s Red List of Threatened Species has classified the Italian hare as 'vulnerable', because it is considered to have a high risk of extinction in the next decade (Rondinini et al. 2013).

A first important step towards the conservation of the Italian hare dates back to 2001, with the publication of the Italian Action Plan for *L. corsicanus*. This plan provides all the available information on distribution, status and limiting factors of this species (Trocchi & Riga 2001). The plan also stresses the improvement of knowledge on the biology and ecology of the Italian hare as important key factors for its conservation.

In recent years, there has been an increasing amount of literature on different aspects concerning this lagomorph, such as its morphometric and morphological characteristics (Palacios 1989, 1996; Riga et al. 2001), its population genetic structure and phylogenetic relationships (Pierpaoli et al. 1998, 1999), its distribution range (Angelici & Luiselli 2001), its health status (Dantas-Torres et al. 2011), etc.

An understanding of the feeding habits of the Italian hare is also essential to identify potential factors influencing the population viability of this taxon, as well as for protection of its elective habitats. However, to date, few studies on the feeding habits of the Italian hare have been conducted. A first contribution describes the diet of the Italian hare from Sicily (De Battisti et al. 2004), where the species is quite widespread and does not appear to be threatened (Lo Valvo et al. 1997). In continental Italy, an early description of the feeding habits of this species is that of Trocchi & Riga (2005). More recent studies (Freschi et al. 2014a, 2014b) have been carried out in in the Regional Park of Gallipoli Cognato Piccole Dolomiti Lucane, which, since 2006, has joined a conservation initiative that aims to recover the Italian hare in the Basilicata region (south of Italy).

One of the aspects of diet yet to be exhaustively addressed is that concerning the selection of food items in accordance with their seasonal availability in the environment (Freschi et al. 2014a). Therefore, in the present study, carried out in the same protected area, we evaluated the seasonal feeding ecology of the Italian hare.

METHODS

Study area

For pellet sampling, a research study area (Fig. 1) which spans approximately 1.78 km^2, with altitudes ranging from 610 (northern slopes) to over 900 (southern slopes) m above sea level, was chosen within the Park (headquarter coordinates: 40°30′49.65″N, 16°8′35.70″E). The mean annual air temperature of the northern slopes is approximately 4 °C lower than that of the southern slopes (11 vs 15 °C). The average annual rainfall varies from 671 mm in the northern slopes to 910 mm in the southern slopes. May and November are the wettest months generally across the area, whereas the warmest months, July and August, are also the driest.

The vegetation of the site includes a wide mosaic of grasslands of secondary origin, spread thickets of dwarf bushes (e.g. *Crataegus monogyna*, *Prunus spinosa*, *Pyrus amygdaliformis*, *Phyllirea*

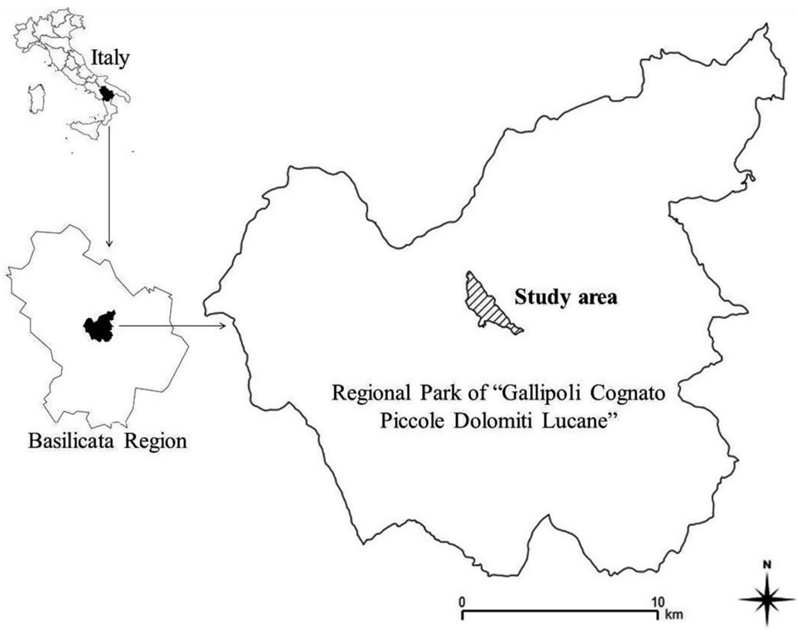

Fig. 1. — Map showing the location of the study area within the Regional Park Gallipoli Cognato Piccole Dolomiti Lucane (southern Italy).

latifolia) and few oaks (mainly *Quercus virgiliana*). The grasslands are characterised by a very high species density. These communities are dominated by hemicriptophytes, but, in the driest conditions, they may be also characterised by several chamaephytes. Among the plants more typically found in the ground vegetation, we mention *Brachipodium* sp., *Bromus* sp., *Carex* sp., *Sanguisorba minor*, etc. Overall, the vegetation of this site is mainly peculiar to the habitat 6210 Semi-natural dry grasslands and scrubland faces on calcareous substrates (Festuco-Brometalia; Habitats Directive, 92/43/EEC).

The exclusive presence of the Italian hare in this study area was ascertained by monitoring activities carried out by the Park (e.g. captures, total censuses, DNA analysis, etc.) within the aforementioned conservation initiative. At the time of the current study, the index of occurrence of the Italian hare in the site was 14 hares/km^2.

Collection and processing of faecal pellets

Collection of fresh faecal pellets took place from December 2011 to November 2012 along eight replicate transects (2 × 200 m), which were separated from one other by ~ 100 m, and spatially distributed throughout the study area. Pellets were collected monthly in each transect from different droppings. From each collection, a minimum of six pellets, of various sizes and formats, were mixed to form a single composite sample. Throughout the year, 96 samples were analysed (eight per month).

The processing of faecal pellets followed the method described by Paupério & Alves (2008), with some modifications. Briefly, each composite sample was first ground in a mortar and then cleared in a 0.05 M solution of sodium hydroxide (NaOH) for 2 hr. Thereafter, it was washed with distilled water over a 63-µm sieve, and the retained material was collected over filter paper, dried and mounted in glycerol gelatine on five microscope slides. Finally, in each slide, the first 10 non-overlapping plant fragments were counted in systematic transects across a slide along alternate rows. A total of 400 fragments were recorded in each month.

Diet composition analysis

Diet composition was determined by the micro-histological identification of indigestible plant fragments recorded in each slide (Baumgartner & Martin 1939; Dusi 1949). Despite its recognised limitations related to differential digestibility of plant material (Holechek et al. 1982), this method is widely used to investigate food habits in different herbivores. Moreover, it is particularly useful for endangered species, since it does not interfere with the behaviour of the animals and does not require handling/collecting/killing individuals. Therefore, given the threatened status of the Italian hare, we considered this method the most appropriate for studying its food habits.

Identification of plant species was carried out by comparing the different features and dimensions of the epidermal cells and other valuable taxonomical structures (e.g. trichomes and stomata form) of the recovered fragments with those of a plant reference material prepared (methods described by Maia et al. 2003) by collecting monthly leaves, stems, flowers and fruits of the plants found in the study site. This reference material (136 plant species) is available at the Laboratory of Environmental and Applied Botany, University of Basilicata. Images of identified fragments were also acquired with a Leica EC3 digital camera (Leica Microsystems, Bannockburn, IL, USA) linked to software for image analysis (Leica LASV4.1).

The taxonomic nomenclature of the identified taxa follows Conti et al. (2005). The fragments that were not identified to the species level were classified as 'unidentified', and were not included in our data set.

Statistical analysis

Monthly data were summed up to obtain seasonal and annual amounts of identified plant taxa fragments. Seasons were defined as spring (1 March–31 May), summer (1 June–1 August), autumn (1 September–30 November), and winter (1 December–29 February). Seasonal and annual values were used to calculate the relative percentage (*rp*) of a taxon by season and year (i.e. annual consumption), respectively,

$$rp = (n/N) \times 100,$$

where n is the number of identified fragments attributed to a given taxon in a given season (or in the year); N is the total number of identified fragments in that given season (or in the year). The above formula was also applied to calculate the following seasonal relative percentages of unidentified fragments: 10.63% (spring); 10.48% (summer); 9.77% (autumn); 12.37 (winter).

Numerical abundances of identified taxa were also analysed through non-parametric multivariate techniques using the Primer v6 software (Clarke & Gorley 2006). A similarity matrix was constructed by means of the Bray–Curtis similarity coefficient by first applying fourth-root transformation on species abundance to downweight the contribution of the most abundant species. From this matrix, an ordination of the samples of each season was performed by means of nonmetric multidimensional scaling (n-MDS) with cluster overlay. A measure of goodness of fit of the n-MDS ordination was given by the stress value. A low stress factor (< 0.2) corresponds to a good ordination with no real prospect of a misleading interpretation (Clarke & Warwick 2001). Analysis of similarities (ANOSIM) was then applied to the Bray–Curtis similarity matrix using 9999 permutations to test for statistically significant differences in diet composition between samples collected in each season. The contribution of each taxon to the average dissimilarity between seasons was calculated using the similarity percentage analysis (SIMPER) procedure in PRIMER. A detailed description of the aforementioned statistical procedures can be found in Clarke (1993).

RESULTS

Diet composition

A total of 73 plant taxa were identified in the faecal pellets of *L. corsicanus* (Table 1). The overall ingestion rate (i.e. annual consumption) ranged from 0.01 to 7.98%. Over half of the taxa (44 of 73) were ingested in low percentages (< 1%), and

Table 1.

Relative percentages of plant species identified in faecal pellets.

Taxa	Seasons				Annual consumption
	Spring	Summer	Autumn	Winter	
Achillea collina	0	0.08	1.27	0.37	0.54
Aegilops geniculata	0.20	0.00	0.00	1.80	0.68
Agrimonia eupatoria	0	0.42	0.13	0.02	0.11
Allium subhirsutum	5.12	3.46	7.21	6.23	5.91
Allium triquetrum	7.31	1.67	0.95	2.71	2.83
Arabis collina	0	0	0.08	0	0.02
Bellevalia romana	2.42	0.84	0.63	0	0.75
Bellis perennis	0	0.04	0.02	0.03	0.02
Brachypodium pinnatum	0	1.25	1.16	0.68	0.79
Brachypodium sylvaticum	21.41	8.05	3.89	4.77	7.98
Bromus racemosus	0	1.90	0.02	0.21	0.37
Buglossoides purpurocaerulea	0	0.04	0.13	0.29	0.15
Capsella bursa pastoris	0	0.11	0.02	0.58	0.23
Carex distachya	2.55	2.70	1.25	5.26	3.15
Carex flacca	2.77	2.47	2.64	3.41	2.91
Carpinus orientalis	0	0.23	0.21	0	0.10
Centaurea solstitialis	0	0.76	0.13	0.03	0.17
Cichorium intybus	0	0.80	0.76	0.76	0.63
Cirsium strictum	0	1.48	3.64	4.83	3.09
Colchicum neapolitanum	0	1.29	1.88	3.16	1.91
Crataegus monogyna	0	0.19	2.12	1.65	1.28
Cynodon dactylon	0.03	1.67	2.66	1.95	1.78
Cynosurus echinatus	0	0.23	0.13	0	0.08
Cytisus hirsutus	0	0.04	0.83	1.36	0.75
Cytisus villosus	0	1.52	0.15	0	0.28
Dactylis glomerata	0	1.14	0.78	1.04	0.79

(*Continued*)

Table 1.

(*Continued*)

Taxa	Seasons				Annual consumption
	Spring	Summer	Autumn	Winter	
Daucus carota	0	0	0	0.03	0.01
Dianthus vulturius	0	0.30	0	0	0.05
Eryngium campestre	0	0.34	0.09	0	0.08
Festuca arundinacea	1.83	5.43	5.97	4.87	4.75
Festuca heterophylla	7.18	4.14	0.97	1.70	2.83
Fraxinus ornus	0	0	0	0.36	0.13
Gagea lutea	2.09	0.99	0.15	0.06	0.60
Geranium dissectum	0	0	0	0.24	0.09
Gladiolus italicus	0	0.23	0.06	0	0.05
Hermodactylus tuberosus	2.55	0	3.58	2.60	2.49
Hypochoeris achyrophorus	0.39	0.65	0.08	0.11	0.23
Lathyrus digitatus	0	0.19	0.23	0	0.10
Lathyrus jordanii	0	0.04	0.27	0.02	0.09
Lathyrus venetus	0	0	0.19	0.02	0.06
Leopoldia comosa	6.23	2.81	0.13	0.05	1.61
Lolium perenne	0	3.50	5.73	1.46	2.82
Lolium rigidum	1.93	1.82	0.40	1.93	1.44
Luzula forsteri	3.69	1.29	2.09	0.63	1.73
Malus sylvestris	0	0.84	1.19	0.89	0.82
Melica ciliate	0	0.68	1.25	0	0.49
Muscari atlanticum	2.09	1.82	0	0	0.65
Muscari commutatum	0.03	0	0	0	0.01
Muscari neglectum	2.35	1.18	0.09	0.11	0.67
Olea europaea	0	0.04	0.02	0.32	0.13
Ornithogalum excapum	0	0.72	0.87	1.05	0.76
Phlomis herba venti	0	0	0.04	0	0.01
Picris hieracioides	1.37	5.74	4.21	2.68	3.39
Plantago lanceolata	2.97	1.67	2.60	2.24	2.39
Plantago serraria	6.40	1.75	2.35	1.25	2.59
Poa trivialis	0.91	1.25	1.38	4.91	2.55
Prunella vulgaris	0.16	0.19	0.55	0.50	0.41
Prunus spinosa	0	5.93	10.22	5.47	6.02
Pyrus piraster	0	1.90	3.41	3.89	2.74

(*Continued*)

Table 1.

(*Continued*)

Taxa	Seasons				Annual consumption
	Spring	Summer	Autumn	Winter	
Quercus cerris	0	0.15	0.15	0.03	0.08
Ranunculus repens	0	0.04	1.38	1.83	1.09
Romulea bulbocodium	6.69	3.91	0.27	1.43	2.39
Rosa canina	0	1.18	1.29	5.66	2.61
Sanguisorba minor	0	0	0.02	0.11	0.05
Sesleria autumnalis	2.94	1.98	0.63	0.39	1.16
Silene alba	0	0.57	0.46	0.08	0.26
Sorbus torminalis	0	1.22	1.90	2.56	1.69
Spartium junceum	0.03	0.19	0.11	0.06	0.09
Stachys officinalis	0	0	0.46	0	0.14
Thymus longicaulis	2.28	1.29	0.53	0.37	0.90
Trifolium angustifolium	0.07	0	0	2.04	0.75
Trifolium pratense	0.98	10.41	11.99	4.82	7.20
Trifolium stellatum	3.04	1.25	0	2.04	1.47
Total	100	100	100	100	100

represented 14.19% of the annual diet. The most consumed species were *Brachypodium sylvaticum* (7.98%), *Trifolium pratense* (7.20%), *Prunus spinosa* (6.02%), *Allium subhirsutum* (5.91%) and *Festuca arundinacea* (4.75%). Altogether, these six taxa accounted for 31.86% of the hare's diet.

The relative percentages of some taxa (e.g. *Rosa canina*, *Pyrus piraster*, *Cirsium strictus*, *Poa trivialis*, *Colchicum neapolitanum*, *Sorbus torminalis*, *Ranunculus repens*, *Ornithogalum excapum*, *Buglossoides purpurocaerulea*) progressively increased from spring to winter. Conversely, the consumption of *Leopoldia comosa*, *Luzula forsteri*, *Thymus longicaulis* and *Sesleria autumnalis* progressively decreased from spring to winter. For some taxa, the relative percentages progressively increased until summer (e.g. *Prunella vulgaris*, *Spartium junceum*, etc.) or autumn (e.g. *T. pratense* and *F. arundinacea*) but decreased thereafter. By contrast, the opposite trend was observed for certain taxa (e.g. *F. heterophylla*, *A. triquetrum*, *Romulea bulbocodium* and *Lolium rigidum*), since their relative percentages progressively decreased until autumn but increased in winter.

The smallest number of taxa (31) was detected in the faecal pellets collected in spring. All these taxa were shared with other seasons, with the exception of *Muscari commutatum* (0.03%). The most consumed species was *B. sylvaticum* (21.41%), followed by *A. triquetrum* (7.31%), *F. heterophylla* (7.18%), *R. bulbocodium* (6.69%) and *Plantago serraria* (6.40%). Altogether, these five plants constituted almost half of the hare's spring diet (48.99%). The remaining 26 taxa were minor contributors to the spring diet, with rates ranging from 0.03 (e.g. *Cynodon dactylon*, etc.) to approx. 5–6% (e.g. *L. comosa* and *A. subhirsutum*).

Sixty-one taxa were identified in the summer diet, and only one, *Dianthus vulturius* (0.30%), was not shared with other seasons. Of all the plant species we found in this season, *T. pratense* and *B. sylvaticum* were the most consumed ones (10.41 and 8.05%, respectively). These plants, along with *P. spinosa* (5.93%), *Picris hieracioides* (5.74), *F. arundinacea* (5.43%) and *F. heterophylla* (4.14%), accounted for 39.70% of the summer diet.

The highest number of taxa (64) was detected in the autumn diet. Among them, *Arabis collina* (0.08), *Phlomis herba venti* (0.04) and *Stachys officinalis* (0.46%) were found only in this season. The following six taxa accounted for 45.33% of the autumn diet: *T. pratense* (11.99%), *P. spinosa* (10.22%), *A. subhirsutum* (7.21%), *F. arundinacea* (5.97%), *Lolium perenne* (5.73%) and *P. hieracioides* (4.21%).

For each of the 59 taxa identified in the winter diet, the ingestion rate was always less than 7%. Among the most consumed species, we note that *A. subhirsutum* (6.23%), *Rosa canina* (5.66%), *P. spinosa* (5.47%), and *F. arundinacea* (4.87%). *Daucus carota* (0.03%), *Fraxinus ornus* (0.36%) and *Geranium dissectum* (0.24%) were found only in the winter diet.

Seasonal variation in dietary diversity

As shown in Table 2, a small number of shared taxa was observed when comparing the spring diet with the other diets: values ranged from 26 (spring vs autumn) to 28 (spring vs winter). Conversely, over three quarters (79.45%) of all taxa were shared by summer and autumn diets. When comparing winter diet with autumn and summer diets, the rate of shared species was 72.60 and 69.86%, respectively.

Fig. 2 shows the results of n-MDS ordination with cluster overlay at a 65% similarity level. The generated two-dimensional stress value was 0.17, indicating a potentially useful representation of the data (Clarke & Warwick 2001; McCune & Grace 2002). In this figure can be seen a clear separation of two main groups of samples. Most of the spring samples and some of the summer samples are similar,

Table 2.

Summary results of taxa observed in each season and shared by seasons.

Seasons		Taxa observed in season A		Taxa observed in season B		Taxa shared by seasons	
A	B	*n*	%	*n*	%	*n*	%
Spring	Summer	31	42.47	61	83.56	27	36.99
Spring	Autumn	31	42.47	64	87.67	26	35.62
Spring	Winter	31	42.47	59	80.82	28	38.36
Summer	Autumn	61	83.56	64	87.67	58	79.45
Summer	Winter	61	83.56	59	80.82	51	69.86
Autumn	Winter	64	87.67	59	80.82	53	72.60

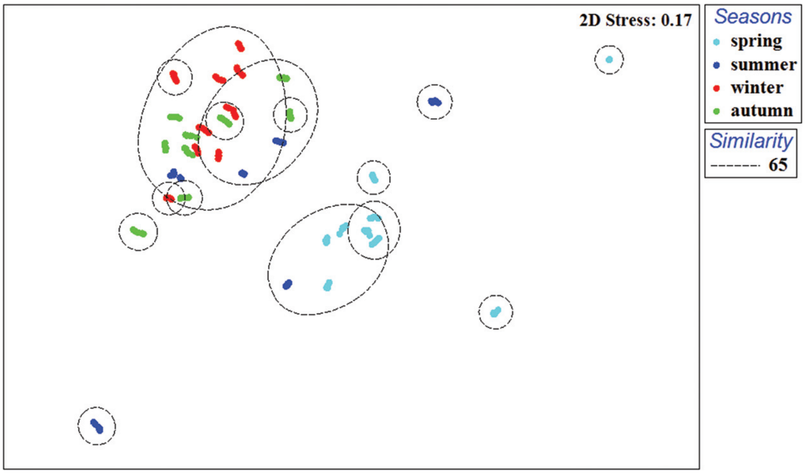

Fig. 2. — Nonmetric multidimensional scaling (n-MDS) plot showing the similarity among seasonal samples, created using the Bray–Curtis resemblance matrix of species abundance data (fourth-root transformed). Overlaying clusters were defined at a 65% similarity level.

and form one group. Another group is formed by the winter samples and by most of the samples collected in the autumn season. This group is also formed by some summer samples.

The ANOSIM test detected significant differences among seasons (global $R = 0.467$, $P = 0.001$), and all pairwise comparisons of seasonal differences were significant (Table 3). The greatest differences in diet composition occurred between spring and autumn ($R = 0.7482$, $P = 0.001$), and between spring and winter ($R = 0.7398$,

Table 3.

Summary results of one-way analysis of similarities (ANOSIM) analyses based on the Bray–Curtis resemblance matrix of species abundance data (fourth-root transformed).

Comparison	*R*-value[a]	*P*-value
Season	Global *R* = 0.467	0.001
Spring vs summer	0.4216*	0.001
Spring vs autumn	0.7482**	0.001
Spring vs winter	0.7398**	0.001
Summer vs autumn	0.2146	0.001
Summer vs winter	0.3334*	0.001
Autumn vs winter	0.2866*	0.001

[a] The pairwise *R* values give absolute measure of how separated the seasons are. * $0.5 > R \geq 0.25$ = overlapping but somewhat different; ** $0.75 > R \geq 0.5$ = overlapping but different; *** $R \geq 0.75$ = well separated; $R < 0.25$ = insufficiently different (Clarke & Gorley 2006).

P = 0.001). By contrast, the smallest difference was found between summer and autumn (R = 0.2146, P = 0.001). The pairwise test also showed a moderate level of similarity between spring and summer (R = 0.4216, P = 0.001).

The contributions of the most representative species to the seasonal dissimilarity of the hare's diet (i.e. SIMPER analysis) are presented in the supplemental online material (Tables S1–S6). Comparison of the spring and autumn diets showed an average dissimilarity of 71.22% with 44 taxa contributing 90.37% to the differentiation: the most important ones were *P. spinosa* (4.86%), *T. pratense* (4.40%), *L. perenne* (4.02%) and *C. strictus* (3.83%; Table S1). The spring and winter diets showed an average dissimilarity of 69.82% (Table S2), with 42 taxa being responsible for 90.96% of the differentiation. Among them, we find *R. canina* (4.17%), *C. strictus* (4.08%), *P. spinosa* (3.82%) and *C. neapolitanum* (3.6%). Forty-four taxa contributed 90.48% of the differentiation found between spring and summer diets (dissimilarity average = 64.13; Table S3). The species that contribute most to the dissimilarity between the two diets were *T. pratense* (3.86%), *B. sylvaticum* (3.07%), *L. perenne* (2.92%), *A. triquetrum* (2.82%), *P. hieracioides* (2.80%) and *P. spinosa* (2.77%). The remaining comparisons of seasonal diets showed a low average dissimilarity (see Tables S4–S6), with values ranging from 49.34 (autumn vs winter) to 55.52% (summer vs spring). Among other species, *R. canina*, *C. strictus*, *P. spinosa*, *F. arundinacea*, *Poa trivialis* and *T. pratense* appeared to be the most important discriminator ones.

DISCUSSION

The Italian hare has a very diversified diet, consuming plant parts from over 70 species. The number of plant taxa identified is higher than that reported by FRESCHI et al. (2014a, 2014b) for other sites situated within the same protected area. The study site chosen in the current study probably supports a greater richness and diversity of plant species, which would have permitted a broad-spectrum diet of the species. Although a high number of plant species was identified in the faeces of the Italian hare, over half of them were ingested in low percentages. This result is consistent with previous studies on this species of lagomorph (e.g. PAUPÉRIO & ALVES 2008; FRESCHI et al. 2014a, 2014b) reporting that only a small fraction of the identified plants was ingested at high rates.

As for other *Lepus* spp. (e.g. *L. arcticus* in: KLEIN & BAY 1994; *L. californicus* in: URESK 1978; JOHNSON & ANDERSON 1984; HOAGLAND 1992; *L. europaeus* in: FRYLESTAM 1986; CHAPUIS 1990; WRAY 1992; PUIG et al. 2007; KONTSIOTIS et al. 2011; *L. flavigularis* in: LORENZO et al. 2011; *L. granatensis* in: PAUPÉRIO & ALVES 2008; *L. starcki* in: MEKONNEN et al. 2011; *L. t. hibernicus* in: HEWSON & HINGE 1990; TANGNEY et al. 1995; WOLFE et al. 1996; DINGERKUS & MONTGOMERY 2001), the most frequently observed fragments in the Italian hare's faeces belong to herbaceous taxa. Plants like *B. sylvaticum*, *T. pratense*, *A. subhirsutum* and *F. arundinacea* occurred at high relative percentages throughout the year. However, the hare's diet also included the consumption of some high-value nutritive foods, such as fruits of *P. spinosa*, *P. piraster* and *Malus sylvestris*. Similarly, KONTSIOTIS et al. (2011) found the fruits of *Malus* sp., *Pyrus* sp. and *Rubus* sp. to be important contributors to the diet of *L. europaeus* from mountainous areas of northern Greece.

In the present study, we aimed to investigate whether the diet composition of the Italian hare differed significantly among seasons. The aim was achieved by employing statistical procedures that have been largely recommended thanks to their widespread validity and the comparative ease with which they can be understood (Clarke & Gorley 2006). Moreover, these procedures have been successfully applied to investigate dietary composition of different species, such as *Chelonia mydas* (Arthur & Balazs 2008), *Dasyurus maculatus* (Glen & Dickman 2006), *Lutra lutra* (Kloskowski et al. 2013), *Ninox strenua* (Cooke et al. 2006), *Phalacrocorax carbo sinensis* (Emmrich & Düttmann 2011) and *Patella caerulea* (Santini et al. 2005). Arthur & Balazs (2008: 214), in comparing the diets of *Chelonia mydas* from seven sites, defined n-MDS as 'useful in understanding the feeding ecology of other sea turtle populations and addressing issues such as variation between age classes, foraging location, or seasonal variation in feeding ecology'. To our knowledge, few studies have investigated the dietary composition of hares by applying these techniques. In a study on diet selection of *L. europaeus* from snowy mountainous of Australia (Green et al. 2013), some of these techniques allowed a comparison of the assemblages of plant taxa fragments among seasons and years of collection.

By applying these techniques to the Italian hare's diet, we found significant differences in food habits across seasons. These result are consistent with those found in previous studies on other *Lepus* spp. (e.g. Wolff 1978; Hoagland 1992; Dingerkus & Montgomery 2001; Puig et al. 2007; Green et al. 2013), and suggest that the seasonally varying proportions of plants in pellets may be related to such factors as plant phenology, abundance, palatability and nutritional quality.

As revealed by ANOSIM, in spring the diet was significantly different compared to other seasons. The analysis of its composition showed a lower number of identified taxa, most of which belonging to herbaceous plants (e.g. *B. sylvaticum* and *A. triquetrum*). It has been reported that herbivores specialise when resource levels are high, and generalise when they are low (Westoby 1974; Belovsky 1978). Given the abundant supply of food resources available at the site in this season, it can therefore be assumed that hares were specialised grazers on herbaceous plants during spring, probably to fulfil their energy and water requirements. Moreover, this is supported by the optimal foraging theory (MacArthur & Pianka 1966), according to which a species attempts to maximise the use of forage resources to meet its requirements with benefits accruing to its reproductive fitness.

The feeding strategy adopted in spring also occurred in summer, which explains the moderate level of diet overlap that occurred between the two seasons. This pattern of diet similarity is clear in Figure 2 from n-MDS: some summer samples are included in the group formed by spring samples, albeit most of them are far distant from this group. SIMPER results indicated that the dissimilarity between spring and summer was mostly contributed by some herbaceous plants (e.g. *T. pratense*, *B. sylvaticum* and *L. perenne)* whose consumption was quite different between the seasons.

Overall, in summer there was a decrease in consumption of those herbaceous plants previously found in spring. These results agree with those observed in earlier studies on other *Lepus* sp. (Homolka 1982; Chapuis 1990; Wray 1992; Wolfe et al. 1996; Paupério & Alves 2008). In a study on *L. granatensis,* Paupério & Alves (2008) also found in summer a decrease in consumption of grasses complemented by the ingestion of alternative plant groups. According to the authors, this feeding strategy could reflect an attempt to compensate for the lower quality (i.e. lower protein and water content) of some herbaceous plants available in this season, in order to maintain reproductive activity. This explanation may be extended to the Italian hare since, in the current study, the decrease in herbaceous plant consumption was offset by that of a higher number of plant taxa with higher protein content and digestibility (e.g. Compositae and Leguminosae), as well as of some fruits which begin to ripen in this season.

Most of the plants identified in summer were also found in autumn: these two seasons shared over three quarters (> 79%) of all identified taxa. This high overlap is well represented in Fig. 2, and it is also supported by ANOSIM and SIMPER results. The differences observed between the two seasons are due not only to the ingestion of new taxa (e.g. *Hermodactylus tuberosus*), but also to the variation in consumption of taxa shared by both seasons. For instance, the consumption of fruits (e.g. *P. spinosa*, *P. piraster*, *Sorbus torminalis*, etc.) was higher in autumn than that in summer, as consequence of a greater availability of ripe fruits in the site. Similarly, some succulent plants such as *A. subhirsutum* or some graminae (e.g. *F. arundinacea*, *C. strictus*, *L. perenne*) were browsed more in autumn than in summer.

A substantial overlap was also observed between the autumn and winter diets, since their samples along with some summer samples formed a single group in Fig. 2. Once again, the presence of a high number of plants shared by seasons has played a key role in calculating the degree of diet similarity. In contrast, the observed differences are due to the changes in availability of these plants from one season to another, and, hence, to their phenology. Plants like *Poa trivialis* or *T. stellatum* are good examples of such an interpretation: their consumption was low in summer and in autumn due to their senescence; by contrast, in winter these plants were in a regrowth stage, and were particularly rich in highly soluble cell contents (Van Soest 1982). Given their high nutrition and palatability, these plants occurred at high relative percentages in winter samples.

The occurrence of herbage regrowth is strictly related to the weather conditions of the site, which are never so extreme and persistent as those featuring some mountain areas of northern Europe (Hiltunen 2003; Rödel et al. 2004).

In conclusion, the current study confirms the generalistic behaviour of the Italian hare, as this lagomorph displays a varying degree of selectivity depending on temporal resource availability, and complements its diet opportunistically with fruits. The results obtained in this study also point to the importance of some plant taxa (e.g. *B. sylvaticum*, *T. pratense*, *A. subhirsutum* and *F. arundinacea*) as important food sources of the hare's diet. Further work needs to be done to establish whether the presence and availability of these plants in other areas could be helpful for identifying the elective habitats of this species and successfully releasing hares into them. Finally, the results of studies of such type should also be considered when designing the management objectives for nature conservation in habitat such as that described in this study (i.e. 6210). This kind of habitat is recognised by the EU as a priority habitat for high biodiversity and environmental protection because of the occurrence of rare and endangered species (Calaciura & Spinelli 2008). Therefore, in some cases, the conservation initiative of the species could go hand in hand with the protection of habitat.

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SUPPLEMENTAL DATA

Supplemental data for this article can be accessed here

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