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Getting the dietary knowledge to restore a missing species: seasonal diet of Atlas deer *Cervus elaphus barbarus* in Tazekka National Park, Morocco

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Atlas deer *Cervus elaphus barbarus* was reintroduced in Tazekka National Park in 1994 to help restore the natural state in the regions of the Middle Atlas and Rif mountains. A study of its diet in this area was recommended by the National Strategy for ungulates in order to get data to assess the feasibility of a subsequent release. So our aim was to study the diet of Atlas deer and its seasonal variation. Faeces were collected in Atlas during 2013–2014 in Bab Klati 520-ha reserve located in the west of the Tazekka National Park. Faecal samples were micro-histologically analysed based on a reference epidermis catalogue of all existing plants in the reserve. Poaceae species, the main representatives of the herbaceous category, were consumed at 28%, 37% and 43% of the diet in autumn, winter and spring, respectively. In summer, the consumption of Poaceae did not exceed 2%, presumably because of their limited availability. *Pteridium aqualinium* was consumed especially in summer (6%). Shrubs were represented by three main species: *Ulex boivinii*, *Cytisus triflorus* and *Lavandula steochas*. Consumption of *U. boivinii* was high in autumn (41%) and spring (31%) and low in winter (16%) and summer (6%). As for *C. triflorus*, the consumption was maximal in winter (30%), average in autumn (19%) and spring (18%) and lowest in summer (2%). *Lavandula steochas* was consumed mainly in autumn (8%). In the summer, trees were the main components of the diet and were represented by the oak species *Quercus faginea* (61%), *Q. rotundifolia* (13%) and *Q. suber* (5%). Our analysis revealed dramatic changes in the diet of Atlas deer in Morocco from one season to the other, indicating that this animal is able to change its foraging strategy based on its needs, and on the changing availability of various plants in the environment.

Among the Cervidae species that have lived in Africa, Atlas deer, or Barbary stag, *Cervus elaphus barbarus* is the last representative. Endemic to North Africa, this deer has disappeared from Morocco. Like the other North African countries (Algeria and Tunisia), Morocco has decided to restore this species through reintroduction to suitable habitats. For this reason, the royal hunting reserve of Kissarit, located in the Middle Atlas region was chosen for the first reintroduction of Atlas deer in 1989 from animals (one stag and six hinds) captured in Tunisia. Then, as part of the implementation of the master plan for protected areas developed in 1994, Tazekka National Park was chosen as an appropriate environment to rehabilitate this flagship species in an enclosure of 520 ha, from two males and four females of Tunisian origin. Reintroduced in 1994, the Atlas deer is now represented by a population of 66 animals according to a census that we performed using the method of listening to the roaring of the deer (Hamann et al. 2013).

Mammalian wildlife management cannot be efficient unless we are able to identify the specific needs of each species. In this context, knowledge of the diet is an essential element to define ecological niches and understand the spatial and temporal use of trophic resources (Butet 1987). Stenset et al. (2016) reported that, to successfully manage and conserve a species, it is important to understand both the feeding ecology of the species and how it is able to respond to changes in the availability of food resources. Katona et al. (2014) reported that before considering additional supply, managers must evaluate the quality of the forest; because the natural food supply can greatly influence the use of food items. Holechek et al. (1982) reported that knowledge of diet defines species which should be replanted in degraded areas, and reveals overgrazing.

The diet study of Atlas deer in Tazekka National Park was recommended by the National Strategy for ungulates (Cuzin et al. 2007). It would provide:

- useful data for the feasibility study of a subsequent release in the region (an in-nature reintroduction in the Middle Atlas region is to be considered).
- a methodology for monitoring the impact of deer on the vegetation of the reserve, which will allow assessing the acceptable population level that avoids degradation.

In this context, we aimed at studying the diet of Atlas deer and its seasonal variations.

Material and methods

Study zone

This study was performed conducted in the Bab Klati 520-ha reserve, which is part of Tazekka National Park located in the north west of Taza city, Morocco. This reserve belongs to Bab Azhar forest, which is one of the best cork oak forest massifs in Morocco.

The vegetation of the reserve consists primarily of high-altitude cork oak *Quercus suber* (meso-mediterranean and supra-Mediterranean), Holm oak *Quercus ilex* and Portuguese oak *Quercus faginea*. These stands can be pure or mixed. The vegetation is characterized by degraded formations (scrubs) and grasslands. There are generally three vegetation groups (Cuzin et al. 2007):

Group 1. Facing south, it is a forestry vegetation type comprising *Q. suber, Juniperus oxycedrus, Cytisus villosus, Cistus salviifolius, Ulex boivini, Lavandula stoechas, Ampelodesmos mauretanica, Pteridium aquilinum, Festuca triflora* and *Cynosurus elegans*. It covers about 60% of the enclosure and is good for grazing and regeneration of *Quercus*.

Group 2. Facing north, it is a dense forest grove vegetation type with *Q. faginea, Q. suber, Acer monspessulanus, C. villosus, C. salviifolius, U. boivini, P. aquilinum, Briza maxima, F. triflora* and *C. elegans*. It covers about 40% of the enclosure and is in good condition for grazing and regeneration of *Quercus*.

Group 3. In the troughs, it is a vegetation type with *Rubus ulmifolius*. It covers less than 1% of the enclosure and is in good condition.

The deer reserve occupies a range of altitudes between 1020 and 1696 m a.s.l. It is located on a rather steep mountain slope, with many valleys. The deer enclosure is traversed by a network of watercourses. In addition to two installed water troughs, there are two streams (El Ghannaj and El Kafza) with plenty of water throughout the year. There are also several permanent sources such as Ain Ben Ahmed, Merja Touila, Ain Eddour and Ain Bab Lakhlafi.

The main potentially-competing animals in the site are wild boar *Sus scrofa* and wild rabbit *Oryctolagus cuniculus*.

Methodology

The microhistological analysis of epidermal fragments in faeces has been widely used in recent years to study the composition of red deer diets (Shah et al. 2009, Zweifel-Schielly et al. 2012, Ferretti et al. 2015, Parikh 2015). Several other methods exist, but those based on direct observations are difficult and inaccurate because the species is shy and not easy to observe, and the land is rugged and the vegetation is dense. For the latter reason, we rejected direct observations (see also Malan et al. 2012). Rumen and oesophageal fistulae are two alternate methods, but require the sacrifice of the animal. However, this species is protected and its population is small, so we adopted the method of micro-histological faecal analysis. The method assumes that we find in the faeces plant fragments that are characteristic of the consumed plant species (Butet 1987). This technique involves comparing the plant fragments in the faeces with a reference collection of the most important local species (Ferretti et al. 2015).

Preparation of the reference catalogue

Building on a former study (Benabid 1999), we conducted an exhaustive inventory of the study site's vegetation (both perennial and annual species), and we collected deer droppings. We visited the site in different times of the year to collect leaves of recorded species. We collected four or five leaves and buds of plant species (or 4–5 cm of the branch ends for needle species). Then, in order to prevent their dehydration, the plant parts were placed in vials containing a 10% glycerin solution (10 ml of 98% glycerol in 90 ml of water) to which 50% of 60° alcohol and a few drops of formalin were added.

Obtaining leaf epidermis can be achieved through chemical or mechanical separation. The second technique was adopted here because it gives higher quality epidermis in less time (Butet 1987). The sampling technique involves using a scalpel or a razor blade and clamp-type tweezers under a binocular microscope at low magnifications (10×, 20×, 40×). We collected leaf epidermis from the underside. The leaf was placed in a small petri dish with water and each epidermis was separated from the internal tissue using two clamps or a knife (Maia et al. 2003). Epidermis was then placed in a petri dish filled with commercial bleach to get rid of the parenchyma and make the epidermis transparent (Burthey-Mandret and Burthey 1997). Finally, it was placed in a dish containing teepol (a liquid detergent) to be rinsed and rid of bubbles (Rech 2011).

The removed and processed epidermises were placed in glycerin between a slide and cover glass, under a trinocular microscope provided with a camera that is connected to a computer and software controlled. Samples were photographed, described and identified according to a combination of two identification keys (Burthey-Mandret and Burthey 1997, Rech 2011) based on epidermal characteristics such as size and shape of cells, stomata, presence of detector or secretor hairs, arrangement of these various elements, etc.

Faeces sampling

In the sampling strategy, there are three sample levels: pellet groups, individual pellets and plant fragments to identify (Maia et al. 2003).

Maia et al. (2003) concluded that a minimum of four pellet groups is necessary to achieve a reasonable compromise between accuracy and cost. Katona and Altbacker

(2002) proposed collecting at least 10 independent pellet groups. Burthey-Mandret and Burthey (1997) collected 122 pellet groups from a deer population of 245 animals and an area of 2000 ha. In our case, we collected 62 heaps of faeces on a 520-ha reserve and from a population estimated to be 66 individuals. To ensure that the sample is representative, Zweifel-Schielly et al. (2012) collected faeces along straight line transects in the most-used deer habitats considering only the five most evenly spaced in each transect. García-González et al. (2016) collected faeces in all areas of the site. In our case, we searched all over the reserve for faeces ensuring they are distant from each other. Collecting was concentrated in the middle of each season.

Regarding the number of pellets, we took 10 from each heap. Burthey-Mandret and Burthey (1997) confirmed that beyond 10 pellets, diet does not change. Hearney and Jennigs (1983) used only five droppings per heap for deer and roe.

Finally, for the third sampling unit (the number of fragments to be identified), we chose to identify 100 epidermal fragments per dropping as did Malan et al. (2012), Shah et al. (2009) and Parikh (2015).

Faeces collection and storage

Only fresh faeces were collected (Malan et al. 2012, Zweifel-Schielly et al. 2012, Bezard et al. 2015, Ferretti et al. 2015, Ben Mimoun and Nouira 2016, García-González et al. 2016) because this is important for assessing temporal diet changes (García-González et al. 2016). The age of faeces was determined from smell, humidity and presence of mucus (Bezard et al. 2015). Stenset et al. (2016) estimated the age of the faeces on the basis of recent weather conditions in order to better assign the samples to the corresponding season. Aryal et al. (2015) and Bezard et al. (2015) observed and followed the animals and collected fresh samples in order to ensure that faeces belong to both sexes (Bezard et al. 2015); but faeces collected without any observation most likely represent both sexes (Bezard et al. 2015). On the other hand, it is impossible to separate faeces of different individuals (Malan et al. 2012). Therefore, we collected fresh faeces at random, in the expectation that they will represent different individuals and both sexes.

To prevent mould and epidermis alteration in faeces, two procedures are possible: oven drying (at 60°C for 48 h or longer if faeces are bulky) and freezing. Mysterud and Austrheim (2016), Stenset et al. (2016), Bezard et al. (2015) Czernik et al. (2013) and Aryal et al. (2015) have placed their faecal samples separately in labelled plastic bags and they opted for freezing until use. Zweifel-Schielly et al. (2012) opted for oven drying at 60°C for 48 h. García-González et al. (2016) have stored the droppings in acetoacetic formalin until analysis. For each sample, we noted useful information such as number, contact information, date, time and type of environment. In our case, we adopted oven drying for summer samples and freezing for others.

Faeces preparation and epidermis analysis

After rehydration, samples were gently crushed with a spoon under warm water on a 5-mm screen to obtain epidermis. The retained part was then placed in bleach for 3 to 4 h until epidermis became transparent. Burthey-Mandret and Burthey (1997) used bleach and considered that it increases the number of identifiable fragments.

For reading under a microscope, we used two slides for each sample with a grid of five columns. The fragments were placed between slide and cover glass using a little glycerine.

Species frequency calculation procedure

To quantify consumed species found in faeces and calculate their relative abundance, we divided the number of identified fragments of each species by the total number of identified fragments multiplied by 100 (Shah et al. 2009, Ligi and Randveer 2012, García-González et al. 2016).

Statistical analysis

To investigate associations between deer individuals and consumed species on one side and between seasons and consumed species on the other side, and to examine similarities among seasons, individuals and consumed species, we used the correspondence analysis (CA) procedure.

Results

Obtained epidermis quality

To preserve summer samples, we chose oven drying to facilitate transportation by air for laboratory analysis, but for the other samples, we opted for freezing. From our experience, it appears that freezing is the better conservation technique. Frozen samples need only a 30-min moisturizing and give easily identified epidermises; whereas dried samples require soaking in water for several hours (usually overnight) before analysis.

Individual variability

Figure 1–4 show the correspondence analysis of the consumed plant species (those found in faeces) and the pellet groups (likely corresponding to defecations by individual deer) during the four seasons.

Annual diet

Table 1 shows the results of the microhistological faecal analysis which reveals the seasonal patterns and shifts in the annual relative abundance of plants consumed by Atlas deer. On an annual basis, the diet of Atlas deer is composed of 30% herbaceous plants, 46% shrubs and 19% trees (Table 1). The Paplionaceae family represents the largest share of shrubs, with two main genera *Ulex* and *Cytisus*. Lavender comes in the second place among the shrubs consumed. Herbaceous plants are represented mainly by the Poaceae family. Trees are solely represented by the Fagaceae family, particularly Portuguese oak.

In addition to what is presented in Table 1, in the faeces we also found *Cytisus grandiflorus, Trifolium campestre* and *Vicia disperma*. Besides these Papilionaceae species, *Cistus salvifolius* and *Urginea maritima* were also found.

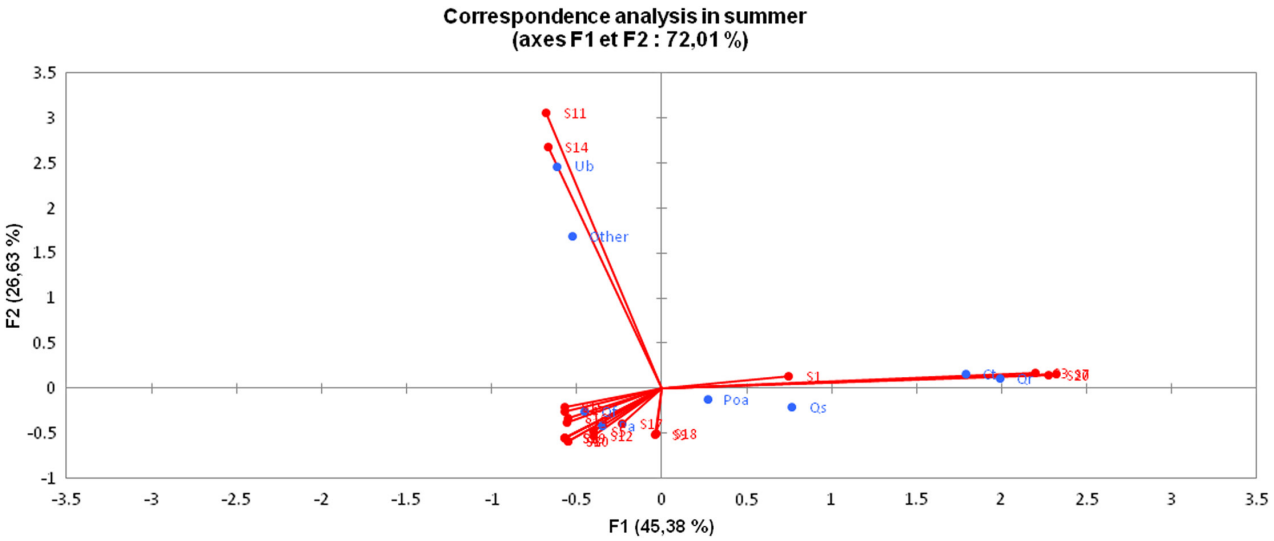

Figure 1. Correspondence analysis of the consumed plant species and individuals of Atlas deer during summer 2013 in Tazekka National Park. Ct: *Cytisus triflorus*, Ls: *Lavandula stochas*, Other: other species, Poa: Poaceae, Qf: *Quercus faginea*, Qr: *Quercus rotundifolia*, Qs: *Quercus suber*, Ub: *Ulex boivini*. S_i (1 to 20): no. of individuals. Blue circles – plant species, red circlls – individuals.

Figure 5 shows the correspondence analysis of the consumed plant species and the season.

Autumn, winter and spring diets

During these three seasons, the results (Table 1, Fig. 2–5) showed a high consumption of shrubs and herbaceous plants. Shrubs' relative abundance was 68%, 57% and 51% during fall, winter and spring respectively. As for herbaceous species, they represented 28%, 38% and 43% of the diet, respectively.

The two families Papilionaceae and Poaceae constituted the largest part of the diet during these three seasons with a relative abundance of 88% in autumn, 83% in winter and 81% in spring. The Papilionaceae family was always the most common, followed by Poaceae.

Ulex boivini represented the largest share in the food spectrum in autumn and spring, but came in second place after the laburnum *Cytisus triflorus* in winter. *Ulex boivini* is a shrub covering 5% of the area (Benabid 1999). It is distributed in space in the form of clumps.

Cytisus triflorus is a shrub measuring up to 1.50 m and covering an average of 37.5% (Benabid 1999). It was planted in clearings in 2002. Lavender *Lavandula steochas* is another shrub that was consumed, especially in autumn and winter.

Summer diet

During summer, the diet changes completely. Results (Table 1, Fig. 1, 5) showed a high consumption of trees, represented by the Fagaceae family, which topped the diet components with an average relative abundance of 79%.

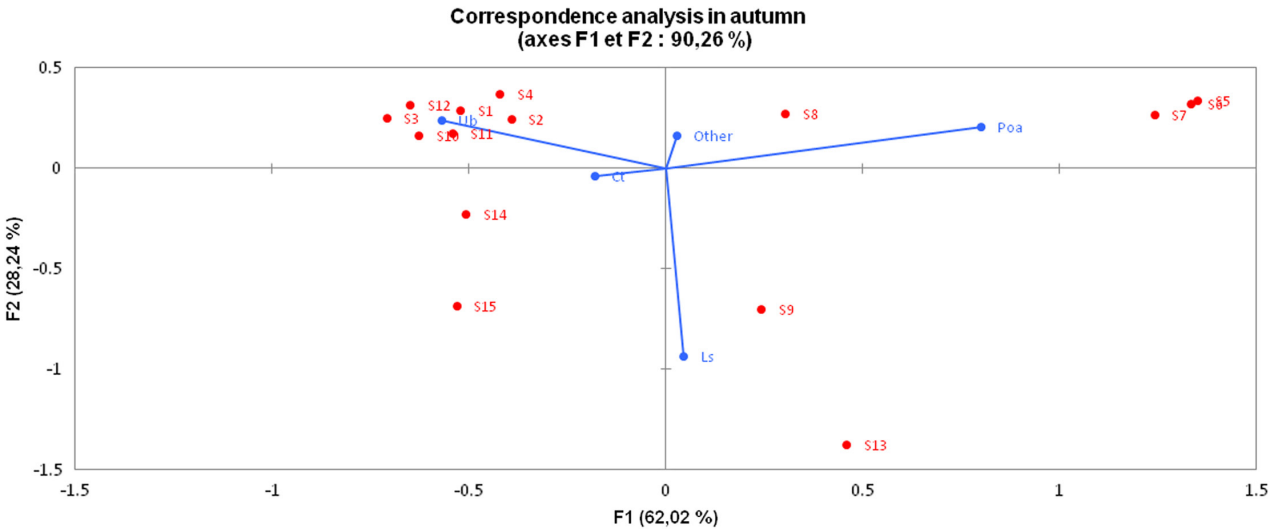

Figure 2. Correspondence analysis of the consumed plant species and individuals of Atlas deer during autumn 2013 in Tazekka National Park. Ct: *Cytisus triflorus*, Ls: *Lavandula stochas*, Other: other species, Poa: Poaceae, Ub: *Ulex boivini* . S_i (1 to 15): no. of individuals. Blue circles – plant species, red circles – individuals.

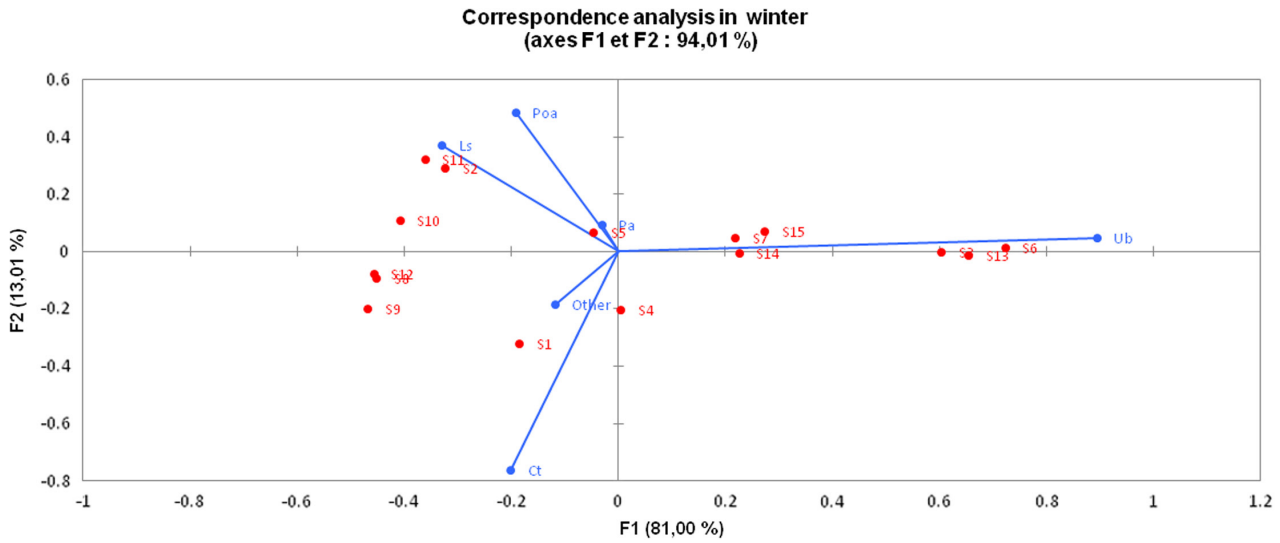

Figure 3. Correspondence analysis of the consumed plant species and individuals of Atlas deer during winter 2014 in Tazekka National Park. Ct: *Cytisus triflorus*, Ls: *Lavandula stochas*, Other: other species, Pa: *Pteridium aqualinium*, Poa: Poaceae, Ub: *Ulex boivini*. S_i (1 to 12): no. of individuals. Blue circles – plant species, red circles – individuals.

Herbaceous plants were led by eagle fern *Pteridium aqualinium*. Shrubs were mainly composed of *Ulex* and *Cytisus*.

Discussion

Individual diet variability

It seems that the diet of Atlas deer varies considerably as shown by the high coefficients of variation (Table 1). In fact, Malan et al. (2012) treated each faecal sample as an independent observation.

During summer

According to the correspondence map (Fig. 1), *Ulex*, *Cytisus*, holm oak and other species contributed most to differences in diet among individuals. They represent 24% of the seasonal deer diet. Portuguese oak and ferns were taken equally by all individuals and did not affect individual variation. Both represent 67% of summer diet. Therefore, at least 67% of the diet during the summer was similar to the entire population. Moreover, 15 out of 20 individuals were grouped in a fairly similar profile (the bottom left quadrant in Fig. 1). So, overall, the diet was rather similar among a rather large proportion of individuals during summer.

It seems that, in summer, deer turn to trees and especially the Portuguese oak (utilized by 70% of the Atlas deer population). This may be explained by the fact that trees, in this period, provide both shade and nutrient-rich acorns. This time of year also coincides with a quasi-absence of herbaceous plants because most Poaceae species are annual, drying and disappearing in summer. The only herb consumed in

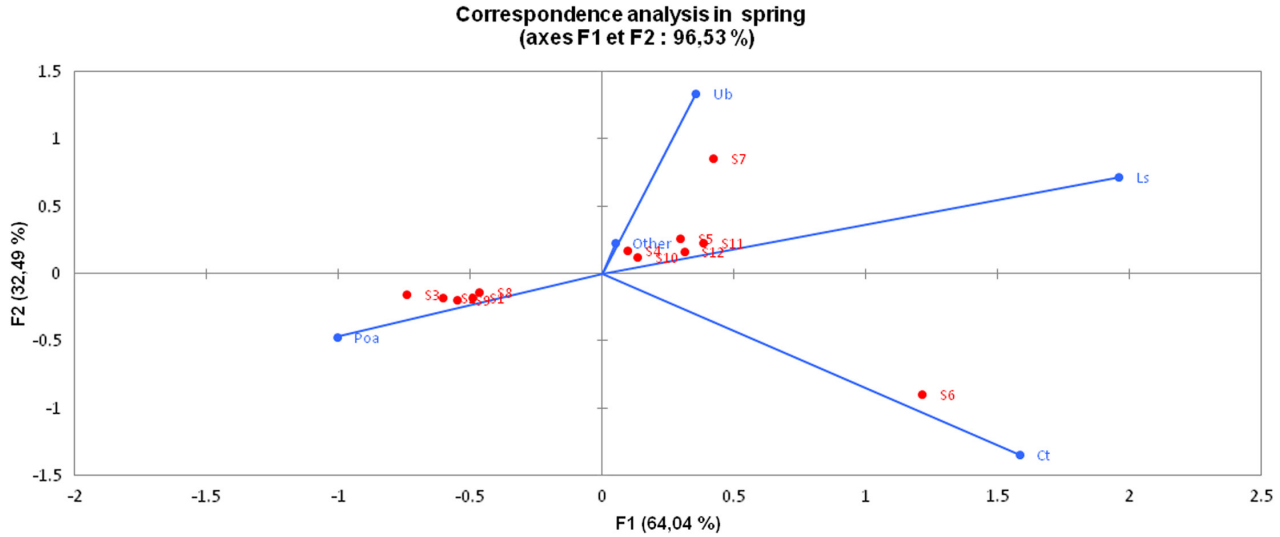

Figure 4. Correspondence analysis of the consumed plant species and individuals of Atlas deer during spring 2014 in Tazekka National Park. Ct: *Cytisus triflorus*, Ls: *Lavandula stochas*, Other: other species, Poa: Poaceae, Ub: *Ulex boivini*. S_i (1 to 15): no. of individuals. Blue circles – plant species, red circles – individuals.

Table 1. Seasonal and annual relative abundance (%) of plants consumed by Atlas deer, *Cervus elaphus barbarus* in Tazekka National Park in 2013–2014. RA = average relative abundance (mean). CV = coefficient of variation (of RA). n = no. of feces heaps analyzed.

			Summer 2013		Autumn 2013		Winter 2014		Spring 2014		Year	
			n = 20		n = 15		n = 12		n = 15		n = 62	
Category	Family	Species	RA %	CV %	RA %	CV %	RA %	CV %	RA %	CV %	RA %	CV %
Shrubs	Paplionaceae	*Ulex boivini*	6	257	41	69	16	91	13	56	23	58
		Cytisus triflorus	2	330	19	60	30	28	18	104	17	58
	Lamiaceae	*Lavandula steochas*	0	0	8	158	11	48	2	135	6	85
Herbaceous	Poaceae		2	300	28	108	37	20	43	59	28	57
	Dennstaedtiaceae	*Pteridium aqualinium*	6	93	0	0	1	45	0	0	2	141
Trees	Fagaceae	*Quercus faginea*	61	54	0	0	0	0	0	0	15	173
		Quercus rotundifolia	13	205	0	0	0	0	0	0	3	173
		Quercus suber	5	133	0	0	0	0	0	0	1	173
Miscellaneous	other species		3	190	2	92	3	54	5	33	3	36
	undetermined		2	54	2	45	2	31	2	36	2	9

large amounts during summer is eagle fern *Pteridium aqualinium*. In addition, *Cytisus grandiflorus* is a deciduous species and has very small leaves. The abundance of the other Papilionaceae species is low, covering less than 1% of the reserve.

During autumn

Lavandula, grasses and other species contributed most to the difference among individuals (Fig. 2). They represent 38% of the seasonal deer diet. *Laburnum*, representing 19% of the diet was taken similarly by all individuals (Fig. 2). From Fig. 2, we can distinguish two groups of individuals: *Ulex* consumers and grass consumers. The former represents 46% of the studied individuals and has 49–84% of *Ulex* in the diet. The latter represents 20% of the individuals and has a diet containing 83–90% of grasses. Thus, in contrast to the summer diet, our analysis reveals that the autumn diet is very different among individuals.

In autumn, with the first rains, the first regrowth of grasses seems to be selected, especially by females (Roberts et al. 2015). Shrubs are also highly eaten when grass availability is limited (Ligi and Randveer 2012). *Ulex* is particularly consumed, more than *Cytisus* and *Lavandula*, probably because of its high nutrient content. The leaves of Portuguese oak dehydrate before their final loss in winter, obliging the deer to change food strategy.

During winter

Ulex boivini (Ub in Fig. 3) is the species most contributing to the differences among individuals. However, it represents only 16% of the diet during winter. Grasses and eagle fern, representing 38% of the diet, are superimposed indicating similar patterns of selection. The remaining 46% is also fairly similar between individuals. So, overall, variation among individual Atlas deer was not high during winter.

Shrubs represent 57% of the diet. The decrease in their percentage, compared to autumn, is due to grass that grows in this period. *Ulex* is consumed, in the first or second place, by about half (at least 40%) of the population. This half is probably composed of males because females consume a diet much higher in grasses, while males consume more trees and shrubs (Roberts et al. 2015). *Laburnum* was differentially taken by individuals. It appears that *Ulex*, despite its thorny character, is energy rich and, in turn, suitable to a

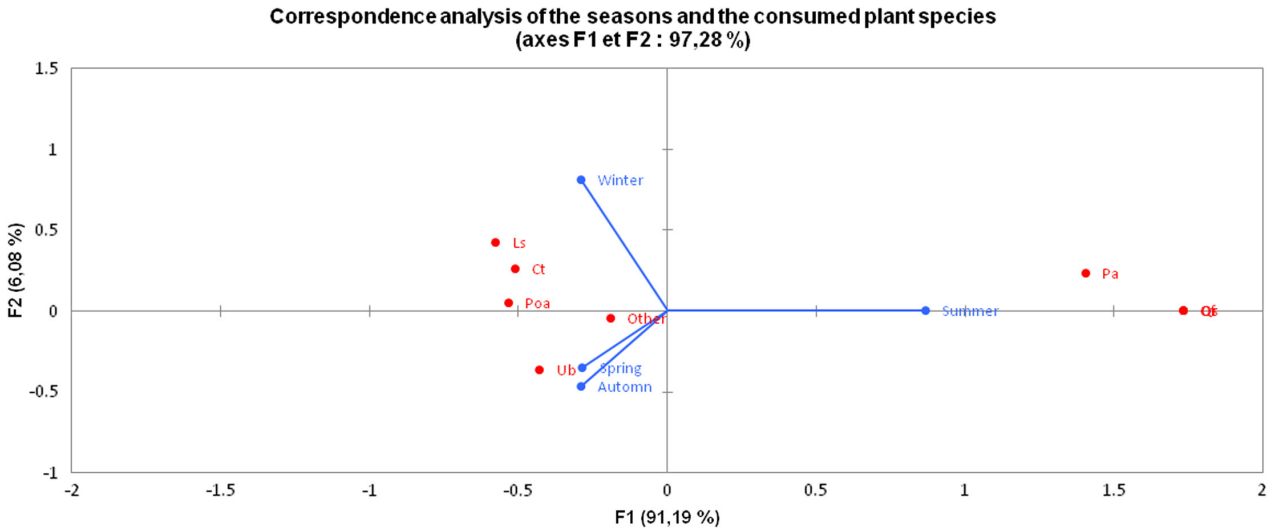

Figure 5. Correspondence analysis of the seasons and the consumed plant species by Atlas deer during the year 2013-2014 in Tazekka National Park. Ct: *Cytisus triflorus*, Ls: *Lavandula stochas*, Other: other species, Pa: *Pteridium aqualinium*, Poa: Poaceae, Qf: *Quercus faginea*, Qr: *Quercus rotundifolia*, Qs: *Quercus suber*, Ub: *Ulex boivini*. Blue circles – plant species, red circles – individuals.

specific group of consumers. Azorit et al. (2012) confirmed that there is only a slight diet similarity between sexes, and the difference may be a consequence of different metabolic requirements.

During spring

As is shown in Fig. 4, *Cytisus* and *Lavandula* contribute most to differences in diet, but they represent only 20% of the deer's diet. Our analysis revealed two main groups of individuals: *Ulex* browsers and grass grazers. The former represent 40% of the population and are probably males. The latter represent another 40% of the population. Their faeces contain 66–78% grass, and are probably females.

It seems that *U. boivini* and *C. triflorus* are quite palatable species, given their percentage in the diet. However, indices are often used to classify species into selected species; avoided and uninteresting species (preference index / Jacobs selectivity index and Petrides index). They take into account relative abundance of the consumed species and their availability on the ground (Lehaire et al. 2014).

The low relative abundance of lavender in spring is likely explained by the fact that the deer turns to a diet rich in grass at this time.

Deer diet plasticity

Our results are different from those obtained by Burthey-Mandret and Burthey (1997) on Atlas deer. These authors showed that the strawberry tree *Arbutus unedo* was at the top of the consumed shrubs with a relative abundance always exceeding 25% whereas that of *C. triflorus* was around 10% in the autumn. In contrast, in our study, *U. boivini* and *C. triflorus* were always leading the consumed shrubs. Burthey-Mandret and Burthey (1997) worked on a reserve of 2000 ha where food resources were probably more diversified than in our 520-ha reserve. Evidently the Atlas deer changes its diet in response to the availability of foods in the environment. This plasticity is an advantage for efforts to re-introduce deer in the areas chosen by the Moroccan national strategy for ungulates established by Cuzin et al. (2007).

There was a significant ($p < 0.05$) dependence between seasons and plant species consumed. Summer was the most different. Autumn and spring had similar patterns. Krojerová-Prokešová et al. (2010) confirmed that the diet in winter differs from the growing season.

Besides season and type of habitat, sex is among the sources of diet variability (Zweifel-Schielly et al. 2012). Azorit et al. (2012) attributed the sex differences to the different metabolic requirements. Roberts et al. (2015) found that females consume more grass whereas males consume more trees and shrubs. Males showed significant differences in habitat use within and between seasons while females showed significant differences only within seasons (Ahmad et al. 2015).

In order to adapt to extreme situations and to efficiently extract nutrients from foods, deer shrink their gastrointestinal tract, which allows it to reduce its appetite, especially in winter, and to avoid wasting time and energy for food search (Arnold et al. 2015).

Conclusion

In conclusion, it appears that the diet of Atlas deer in the reserve Bab Klati located in the National Park Tazekka is not highly diversified. It is mainly composed of grasses and ferns, regarding herbaceous plants, *C. triflorus* and *U. boivini*, representing the most consumed shrubs and oak species for the trees. Our analysis revealed that autumn and spring diets were similar, and that evidently Atlas deer changes its diet based on its needs and what the environment offers.

Knowledge of the deer diet allows understanding how this animal behaves and, in turn, how it will likely affect its environment. We suggest the use of techniques to increase grass resources and thus reduce potential damage to trees as the Atlas deer restoration continues.

Faecal microhistological analysis is a good method for monitoring the seasonal diet of Atlas deer and, possibly, of many endangered species. We recommend monitoring the deer diet for several years, especially after release in nature; and quantifying available plant food resources better so that consumed species can be more rigorously classified as selected, avoided, or taken in proportion to their occurrence.

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