

Allegato 3a_3

DNA Barcoding method to estimate summer diet overlap of Chamois (*Rupicapra, rupicapra*) Ibex (*Capra, ibex*) in the context of climate change impact on mountain ungulate populations in Gran Paradiso National Park.

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Introduction

There is a strong evidence of climate changes impacts and their consequences both on loss of biodiversity at ecosystem scale and on species population dynamics at community level (Walther et al. 2002). A wide range of studies describes different responses on target species due to climate change such as habitat distribution shift, survival rate switch and increase of extinction risk (Thomas et al. 2004). In particular species living in alpine and arctic habitats could be more exposed to climate changes effects (Post et al. 1999). In winter, climate conditions have a substantial impact on ungulate population (Mysterud et al. 2003). Snow depth in ungulates can reduce winter range habitats with direct nutritional effects (White et al. 2009) and increase their locomotion costs (Dailey and Hobbs 1989) with consequent impact on survival rates or the oldest and youngest age classes (Garrott et al. 2003). In summer, pasture home range condition can affect survival rates influencing nutrients assimilations of vegetation consumed and also their availability of selected plants used (Parker et al. 2009). In particular for mountain ungulates summer range characteristics are a trade off between quality and quantity of vegetation (White et al 2011). Moreover, as suggested by Aublet et al. (2009), high temperature during summer can produce health stress reducing foraging rates and selecting habitat with lower quality of vegetation. Understanding such climate changes inferences on species population dynamics and on their habitats is the hardest challenge in ecology field (Walther al. 2002).

The case of ibex (*Capra ibex*) decline in the Gran Paradiso National Park suggests how both winter and summer climate factors mediated by density dependent components can directly and indirectly shape ibex population dynamics (Jacobson, et al. 2004, Pettorelli, et al. 2007, Aublet, et al. 2009, Mignatti, et al. 2012). In particular Pettorelli et al. (2007) highlights how high quality forage availability is a limiting factor of growth and survival of ibex lamb. A fast change in plant productivity due to alteration of winter and spring weather during the green-up could reduce lamb performance (Pettorelli et al. 2007). Those factors associated with constraining effect of temperature on ibex foraging behaviour (Aublet et al 2009) and the spatial and dietary competition with the growing chamois (*Rupicapra, rupicapra*) population present in the Gran Paradiso National Park (Mignatti et al.2012) could explain part of the increase of ibex lamb mortality during summer period and contribute to understand mechanisms shaping ibex population dynamics. Considering those assumptions, understand ibex and chamois diet ecology is the first step to link a potential climate change impact on plant phenology and those feeding behaviours of those mountain ungulate population. Moreover diet overlap together with spatial overlap and resource limitation is one of the condition to asses interspecific competition between ungulates (de Boer and Prins, 1990).

We used Dna barcoding techniques (Taberlet et al. 2007) as non invasive techniques to asses ibex and chamois diet (Taberlet et al. 2007). This method is an alternatives of traditional methods as: direct observation, gut content analysis, and microscope faecal analysis, producing more accurate and robust data i.e: it is possible detect a large number of plant species depending on the reference databes of plant Dna used (Valentini et al. 2009).

The main objectives of this study were: (i) assessing the most frequent plant families driving ibex and chamois diet (ii) estimating dietary overlap and competition of the two species and between two sexes only for ibex (iii) estimating plant associations driving ibex diet ecology in order to focused future research.

Methods

The study was conducted during summer 2013 in Valsavarenche valley, a part of Gran Paradiso National Park (45°26'N, 7°08'E) in Western Italian Alps. The study area altitude ranges between 1600 to 3300 meter above sea level and it is located in the Levionaz area. During 5 sampling periods from May to September, 245 fresh faecal samples were collected for three categories respectively: (i) ibex females (n=95), (ii) ibex males (n=88) and (iii) chamois (n=62) both sexes together. The aim was to

reach at least 12 sample each category each month with the priority for marked individuals for both sexes of Ibex. Chamois population in the area had not marked individuals. The faecal sample collection was designed in order to avoid pseudo-replication of same individual.

A total of 180 faecal samples, divided in 12 sample each category/month, were selected for Dna barcoding analysis. Diet composition at family and species level was evaluated with trnL (UAA) approach (Taberlet et al. 2007) due to its efficiency on deteriorated herbivore faecal samples (Valentini et al. 2009). Diet composition of ibex and chamois was evaluated at family level. Only for ibex, female and male categories was also evaluated diet composition at species level for a more detailed diet descriptions (Bal 2014). Barcoding procedure where the same of Switzerland National Park and performed at SPYGEN[®] laboratory in Grenoble, France. Frequency of occurrence was estimated of each vegetation family sampled (*N° food items*). Morisita (Horn 1966) and Pianka (Pianka 1973) similarity indices with bootstrapping procedure were calculated to estimate diet overlap between the two ungulate species (Shrestha et al. 2005). Moreover Morisita index was calculated on species level with Dna barcoding dataset only for female and male ibex categories in order to reach a more accurate description of plants used and to have a suggestion of particular plant association related to ibex dietary ecology. Ibex diet with this level of analysis were partitioned in two categories early summer (may-june) and late summer (july-september). It was tested differences between the two periods and sexes with chi-squared test (Bal 2014). The analysis was run using spaa package (<http://cran.r-project.org/web/packages/spaa/spa.pdf>) in R (R Development Core Team, 2006).

Results

Dna barcoding at family level showed a low success rate of total analysed samples ranging from a minimum of 45% (female ibex) to a maximum of 55% (male ibex) compared to success rate of total analysed sample at species level of 96% for both female and male ibex. This variation reflected a difference in number of food items each category considered and level of analysis (Table 1). A total of 22 plant families were recorded in ibex and chamois summer diets. Plant family relative importance based on frequency of occurrence shows differences between both species and sexes considered. Female ibex had the lowest number of plant family presence (n=9) followed by male ibex (n=12) and chamois (n= 16). The most important plant families (≥ 0.12) were in decreasing order: (i) female ibex: Fabaceae, Saxifragaceae, Poaceae and Asteraceae, (ii) male ibex: Rosaceae, Saxifragaceae, Poaceae and Fabaceae (iii) chamois: Ericaceae and Fabaceae (Figure 1).

Sample size		Family level			Species level	
Species	Sex	Tot. individuals	N° sampled individuals	N° food items	N° sampled individuals	N° food items
Ibex	M	60	33	49	58	136
	F	60	27	33	58	134
Chamois	Ind.	60	30	46	-	-

Table 1. Description of Dna barcoding dataset sample size at family and genus level of diet analysis for Ibex (*Capra ibex*) and Chamois (*Rupicapra rupicapra*). A total of 180 faecal samples were collected in summer 2013. It is reported: the number of successful sample analysed (*N° sampled individuals*), the number of food items occurrence (*N° food items*) for both family and species level.

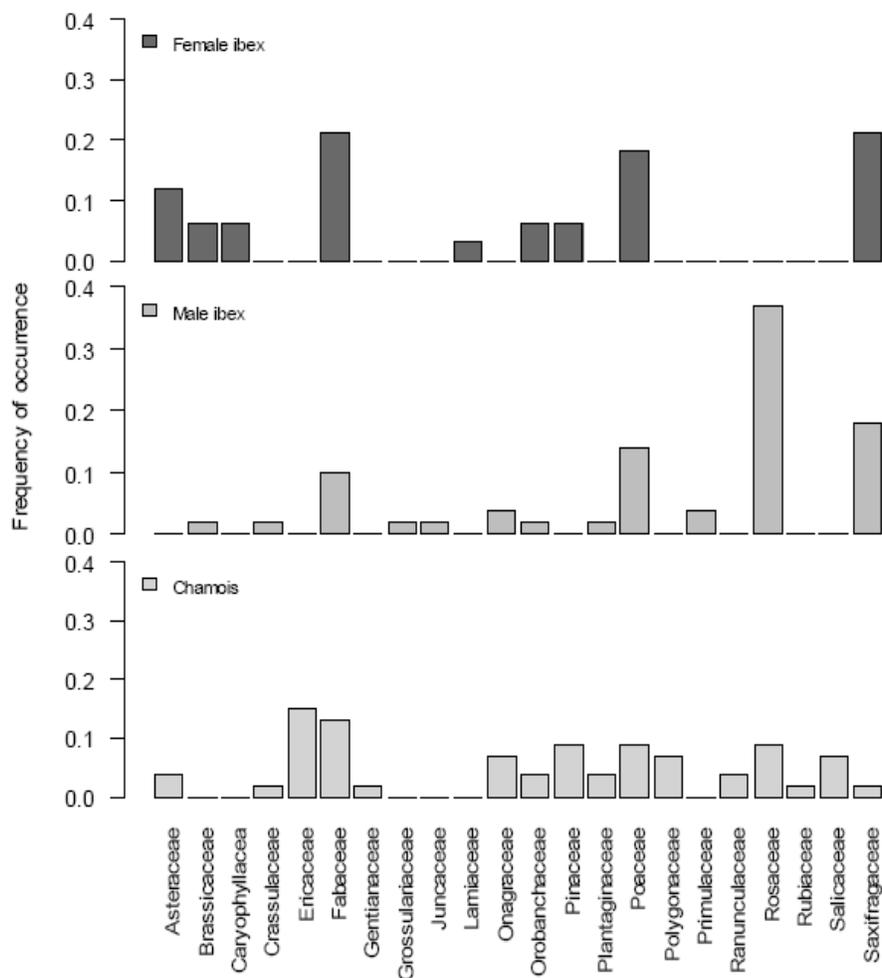


Figure 1. Relative importance of all plant families (n=22) based on their frequency of occurrence in the summer diet of the three considered categories: female ibex, male ibex and chamois in Gran Paradiso National Park. Frequency of occurrence was estimated as the ratio between the number of food items for each plant family and the total food items for each category. Those results are from the Dna barcoding dataset analysed at plant family level.

Dietary overlap estimated with both Morisita and Pianka similarity indices were the most meaningful between female and male ibex. Chamois had the greatest diet overlap with female ibex and the smallest with male ibex. (Figure 2).

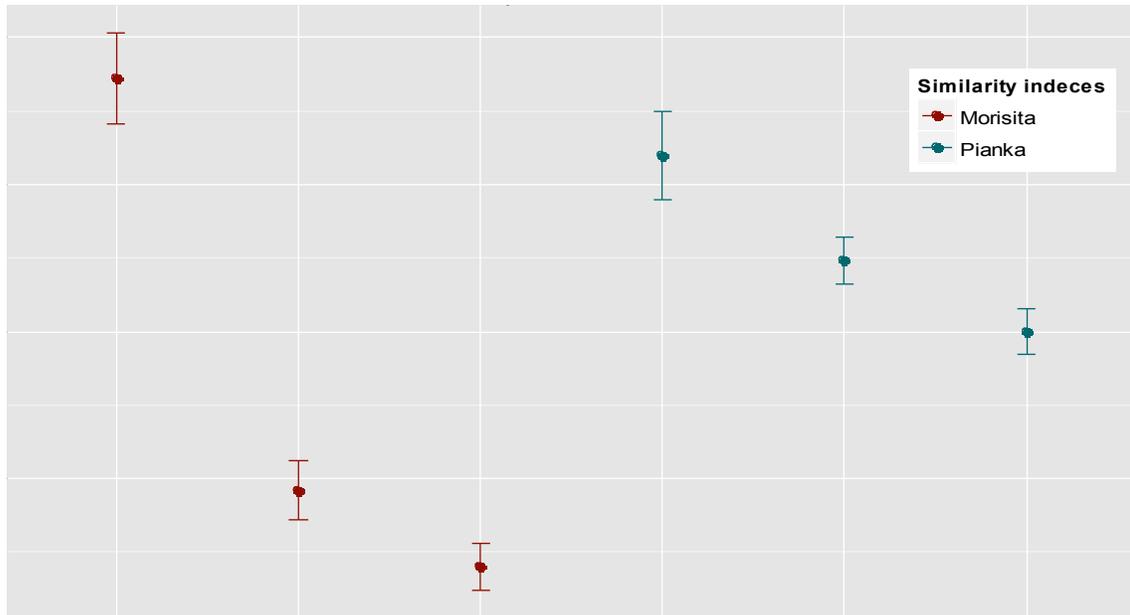


Figure 2. Dietary overlap between ibex (male and female) and chamois at plant family level during summer 2013 in Gran Paradiso National Park. Morisita and Pianka indices bootstrapped values (mean \pm SE) for three considered categories: (i) ibex (F-M), female vs. male ibex, (ii) ibex (F) - chamois, female ibex vs. chamois and (iii) ibex (M) - chamois, ibex male vs. chamois.

Ibex summer diet from Dna barcoding dataset analysed at plant species level described an important variation of plant species used by Ibex. There was a significant difference between female and male ibex diet ($\chi^2 = 57.57$, $P < 0.001$). Cyperaceae and Poaceae were the most used by male ibex while and secondly Cistaceae, Asteraceae and Saxifragaceae by female ibex. Rosaceae was exclusively used by male ibex. Cyperaceae not found at family level of diet analysis, with species level here is with the highest frequency of occurrence (Figure 3).

Morisita diet overlap index of 0.72 calculated without bootstrapping procedure showed a consistent diet overlap (Bal 2014). There was a strong significant difference between spring (May – June) and summer (July – September) ibex diet ($\chi^2 = 34.58$, $P < 0.001$). Fabaceae was found in the spring ibex diet while Orobanchaceae had a substantial increase in the summer period (Figure 4) (Bal 2014)

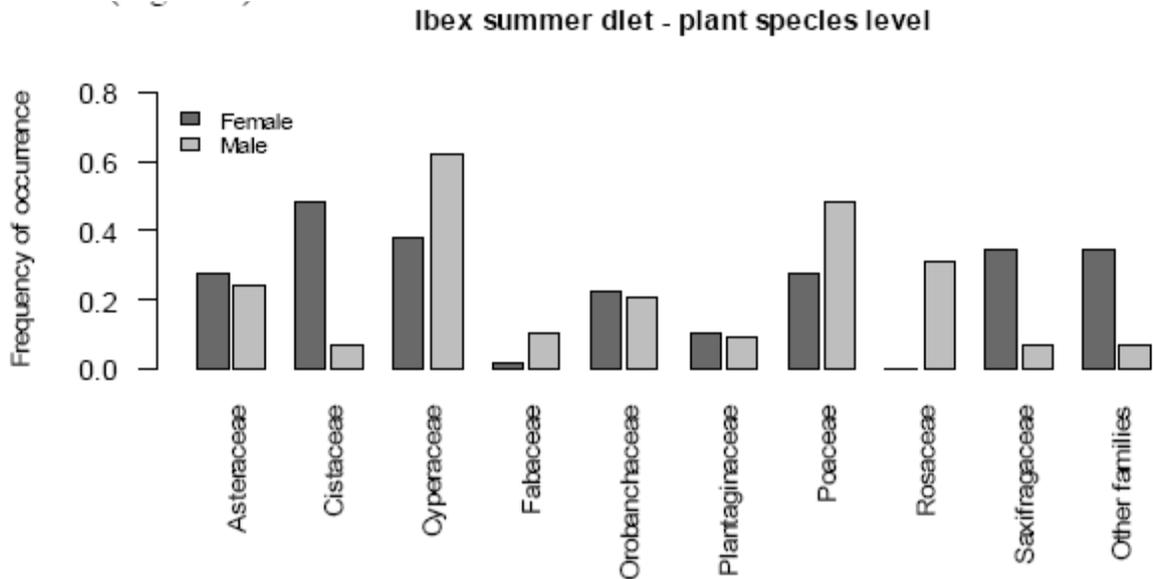


Figure 3. Relative importance of plant families (n=10) based on their frequency of occurrence in the summer diet of female and male ibex, in Gran Paradiso National Park. Families with frequency of occurrence lower than 0.1 were grouped in “Other families” category.

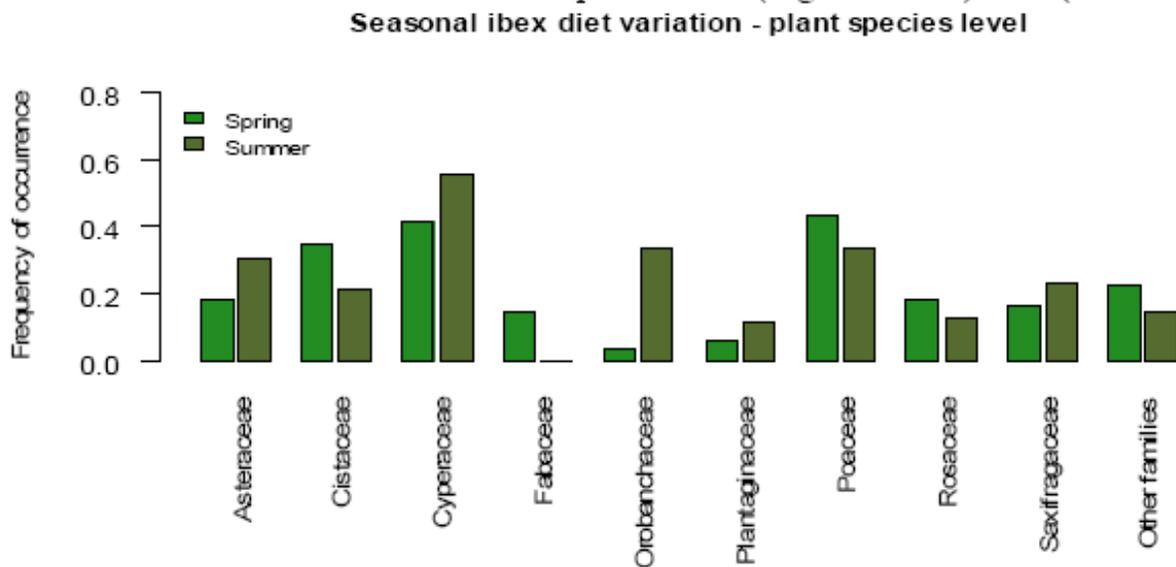


Figure 4. Seasonal ibex diet variation (Frequency of occurrence) at plant species level from DNA barcoding in Gran Paradiso National Park. Families with frequency of occurrence lower than 0.1 were grouped in “Other families” category. Summer diet was divided in two categories: (i) Spring (May – June) and (ii) Summer (July – September).

Ibex showed a frequency of occurrences of plant family depending the periods considered. Female had a considerable increase of Orobanchaceae and Saxifragaceae in the summer period while male had respectively a strong increase of Cyperaceae and a decrease of Poaceae in the summer period (Figure 5)

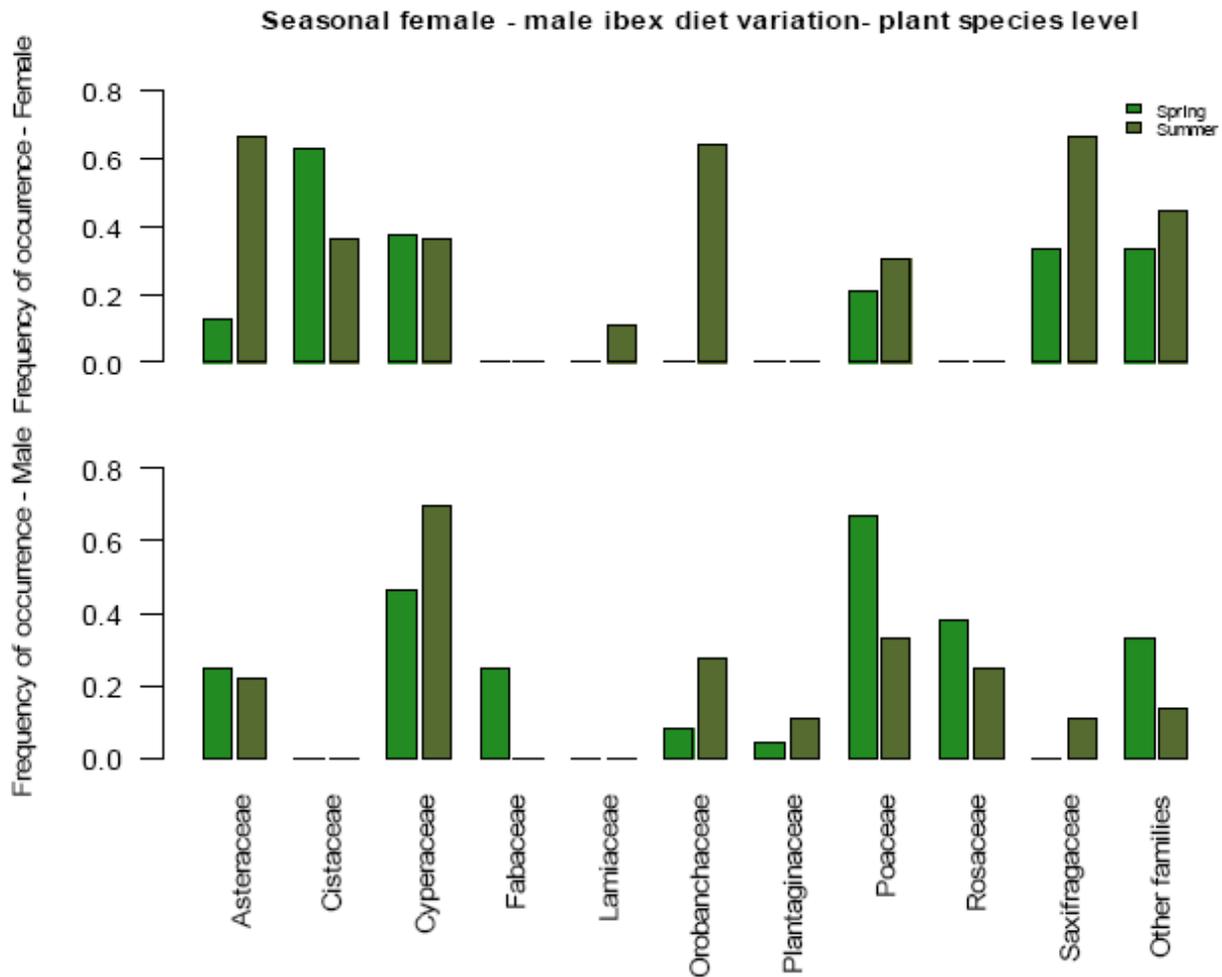


Figure 5. Seasonal ibex diet variation (Frequency of occurrence) at plant species level for female and male ibex from Dna barcoding in Gran Paradiso National Park. Families with frequency of occurrence lower than 0.1 were grouped in “Other families” category. Summer diet was divided in two categories: (i) Spring (May –June) and (ii) Summer (July – September) and ibex grouped for sex classes.

Discussion

This study confirms the power of Dna barcoding approach to assess ungulate diet composition (Valentini et al 2009) and contributes to understand the nature of competitive trophic interactions between ibex and chamois.

The diet analysis at plant family level showed Fabaceae (Leguminosae) and Poaceae (Graminaceae) as the most important plant families present in both species and supporting ibex and chamois as intermediate feeders consuming mostly grasses and forbs if available (Hofmann 1989). However there are relative differences: ibex diet includes Saxifragaceae (both sexes), Asteraceae (female ibex) and

Rosaceae (male ibex) as other main families selected, all of those had a considerable high frequency of occurrence compared other plant families (Figure 3). In female ibex diet, the higher frequency of occurrence of Saxifragaceae and exclusively of Asteraceae plant family can be explained by the sexual spatial and social segregation with male ibex (Bon et al 2001) and the different female ibex summer spatial ecology characterized by the preference of steep and rocky habitats during giving birth and lactating periods as a response to anti-predatory behaviour (Grignolio et al. 2007). The frequency of occurrence particularly high of Rosaceae plant family of male ibex could be related to the use of low altitude pasture during spring and early summer (Grignolio et al. 2003). In contrary chamois diet showed a lower differences between plant family consumed: Poaceae, Onagraceae, Pinaceae, Polygonaceae, Salicaceae and Rosaceae frequency of occurrence were barely lower compared the Ericaceae and Fabaceae. This results suggest a different dietary ecology of chamois and partially support previous study assessing that overall Cistaceae, Fabaceae, Asteraceae, Poaceae and Globulariaceae plant families had frequency of occurrence higher the 90% of the total chamois faecal sample analysed (Rayè et al 2011). In this case, due to the high taxonomic level of the analysis (family) and the dna plant reference database used was not possible to detect Cistaceae and Globulariaceae in chamois diet as well as Cyperaceae in ibex diet that were found in the species level of the analysis (Bal 2014).

Diet similarity indices (both Morosita and Pianka) showed an intermediate overlap between the two species, barely stronger for female ibex. Those results required specific effort in order to understand interaction mechanism of those mountain ungulate. Silba (2014) suggested that chamois presence had a negative effect on ibex distribution and a temporal partition of space and trophic resource use between two species could be present at different spatio-temporal scales (Silba 2014). Therefore in this direction future study should be direct.

The diet analysis at species level only for both sexes of ibex, demonstrate the influence of the taxonomic level of analysis on current analysis of faecal samples: Cyperaceae, not found with the previous dna plant reference dataset, here is the family with the highest frequency of occurrence in ibex diet, followed by Poaceae, Cistaceae and Asteraceae (Figure 5). Those findings are similar on what found by Zing (2009) in a similar study in the Swiss National Park even if the diet analysis techniques where different. The significative diet variation during the spring (May –June) and the summer (July-September) reflects the differences in habitat use and spatial behaviour already discussed (Grignolio (2003, 2007). However the variation of frequency of occurrence between female and male suggests a

possible temperature constrain effect influencing foraging strategy (Auble et al. 2009). The increase in summer of Saxifragaceae in ibex female and the decrease of Poaceae and Rosaceae in male ibex could be mediated by temperature and climate variation (Figure 6). Further research is required to investigate those aspects with a longer time interval to increase statistical inference.

This study underlines the importance of the sampling protocol in the field directly affecting the quality of the Dna barcoding results. Following the target animal as closest as possible and collecting faecal sample as fresh as possible with a correct conservation are important points to not underestimate. The Dna plant taxon references database has to be based on previous vegetation survey if possible in order to increase the inference of the analysis (Valentini et al.2009). Rayè et al (2011) showed how to use Dna barcoding to have quantitatively results on ungulate diet. Particular attention should be paid at laboratory level in order to have the analysis procedure as suitable as possible to research specific questions and approach.

In conclusion, this study is indicative of the need of individual based studies on plant associations and mountain ungulates relationship and interactions. As suggested by Ferrari et al. (1988), particular plant families (as Fabaceae) can play an important role in chamois diet ecology. Understanding how those alpine plant families react to climate change and how habitats selected by ibex are shaped by those factors is the key point to understand complex mechanisms behind mountain ungulates in the contest of climate change impact and in a wider perspective be crucial for management and conservation of ibex.

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