Allegato 3a_1

Spatial distribution and space use of ungulate species in Gran Paradiso National Park

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Introduction

Alpine ungulate monitoring needs shared data collections realized with standard methods and scientific protocols to appreciate conservation status: census, mapping methods, GPS-tracking, genetic analysis, capture-mark-recapture, occupancy models and species distribution models (SDM) are useful in monitoring research and wildlife management. Since 1956 ibex and chamois populations have been historical monitored in Gran Paradiso National Park (GPNP) twice a year in July and September. From 1985 ibex and then chamois (1993) census observations have been mapped to monitor the ungulate distribution throughout the years and to assess real area occupied by species and their seasonal and annual fluctuations in spatial distribution. Long-term observation data are extremely valuable for the analysis of ungulate space use patterns if methodology remains the same over the years. Landscape ecology approach is based on the notion that environmental patterns strongly influence ecological processes (Turner 1989) and the purview of landscape pattern analysis allows us to quantify the spatial pattern and their dynamics. Mapping data censuses allow us to appreciate some spatial indexes useful to evaluate spatial pattern distribution throughout the years and their relative trends in long term monitoring. In spatial pattern distribution, especially trend of real occupied area by species is an important index to evaluate conservation status of the species together with trend of ungulate abundance throughout the years. Moreover ungulate census data localisation could allow us to describe distribution pattern and environmental characteristics of real occupied territories and their relative temporal change to evaluate trends, especially in long temporal data series, which could appreciate possible correlation with population dynamic, trophic resource range and abundance, climatic change, or anthropogenic factors. Finally, simple descriptive analysis of ungulate distribution could be a

support to specific research in space use behaviour and trophic resource competition between alpine species and intra-specie sexes.

Methods

Park rangers have monitored alpine ungulate population (chamois and ibex) with the same census methods (block counts) throughout the years. From 1999, only September censuses have been carried out in all GPNP territory (extensive census).

The park area is divided into 36 surveillance zones that can be covered in two days and each Park ranger has an area of average extension of 1100 ha ±185.2 SD.

The rangers count the population by walking over established routes, and they record the number of individuals and distinguish them according to species, sex and age classes.

Ungulate localisation had hand-drawn mapped on IGM (Military Geographical Institute) map (1:25.000) from 1985 to 1998. Since 1999 rangers have referred the ungulate presence localisation on UTM grid overlapped on GPNP territory. Monitoring unit grid is a 250 m X 250 m cell (6.25 ha) and it was produced by dividing into 16 identical parts each UTM kilometre long grid and identified by univocal ID. From 1999 to 2010 ungulate observations were localised using CTR (Regional Territorial Cartography) map (1:10.000) and reporting the ID's grid of observation on census form; instead from 2012 data have been localised directly using GPS referring always on grid unit. For the above-mentioned reason, ungulate localisation data have been more accurate starting from 1999.

Since 2000 rangers have occasionally collected and localised direct (observations) and indirect (scats, tracks) ungulate presence signs (roe deer, red deer, chamois and ibex) throughout year, and from the beginning of the Great Project ungulate presence has been systematically registered.

Ungulate localisation from hand-drawn maps (from 1985 to 1999) got digitalised using GIS software assigning a level of accuracy presence localisation (1= high accuracy; 2= mean accuracy; 3= low accuracy) in order to compare them to recent and more accurate data.

Data digitalisation, data analysis and species presence's distribution map were performed with open source software QGIS 2.2.0. In presence distribution map of ungulate occasional observations, we considered winter season from November to March and Spring-Summer season from May from to September (2010-2014).

The open-source software Fragstats3 (McGarigal et al. 2012) had allowed us to analyse the pattern of alpine ungulate's distribution throughout the years. This kind of analysis is related

to landscape ecology. We have selected indexes referring to class level to understand the configuration of alpine ungulate's patches. We have used total area (TA) and number of patch (NP) as composition metrics to quantify the composition of the map without reference to spatial attributes. We have chosen radius of gyration (RG), Euclidean nearest neighbour (ENN) and patch cohesion index (PCI) as spatial configuration metrics to quantify the spatial configuration of the map with distribution statistics (mean, standard deviation, coefficient of variation) that provide a statistical summaries of the patch metrics. Fragstats3 indexes are described in table 1A in Appendix.

Trends throughout the years have been analysed with Mann Kendal Trend Test: test for monotonic trend in a time series z[t] based on the Kendall rank correlation of z[t] and t.

Topographic and morphological features (elevation, slope, roughness and aspect) of ungulate's observation have been obtained from Digital models (DEMs) TINITALY/01 DEM (Tarquini et al. 2007; Tarquini et al. 2012) with a spatial resolution of 10 x 10 m. Roughness is the largest inter-cell difference of a central pixel and its surrounding cell, as defined in Wilson et al 2007.

Levene Test has been used to evaluate variance distribution between data samples thus to apply Kruskall Wallis Test and so to appreciate significance annual difference in spatial distribution considering topographic and morphological features of grids used by each ungulate species. Not statistical analysis has been performed with red deer data due to small sample size.

For statistical analysis and graphics drawing, we used R (version 3.0.3).

Results

Gran Paradiso National Park ungulates distribution maps are in Appendix (Appendix Fig. 1A-Fig. 18A). We have performed distribution maps with ibex and chamois September census data considering different period: 1985-1992 period; 1993-1999 period; 2000-2006 period; 2007-2013 period and finally 2011-2013 (Fig1A-Fig.9A). Moreover, an overlap distribution map has been done with data census from 2000-2013, where ibex and chamois presence grids coincided (Fig.18A). We have prepared seasonal (winter and spring-summer) distribution maps for each ungulate species (red deer, roe deer, chamois and ibex) considering their direct and indirect signs of presence collected from 2010 to 2014 by rangers (Fig.10A-Fig.17A).

Distribution data analysis for red deer and roe deer is at the moment preliminary, seen their expanding range, and also ibex and chamois winter data analysis could be considered introductory due to sample size.
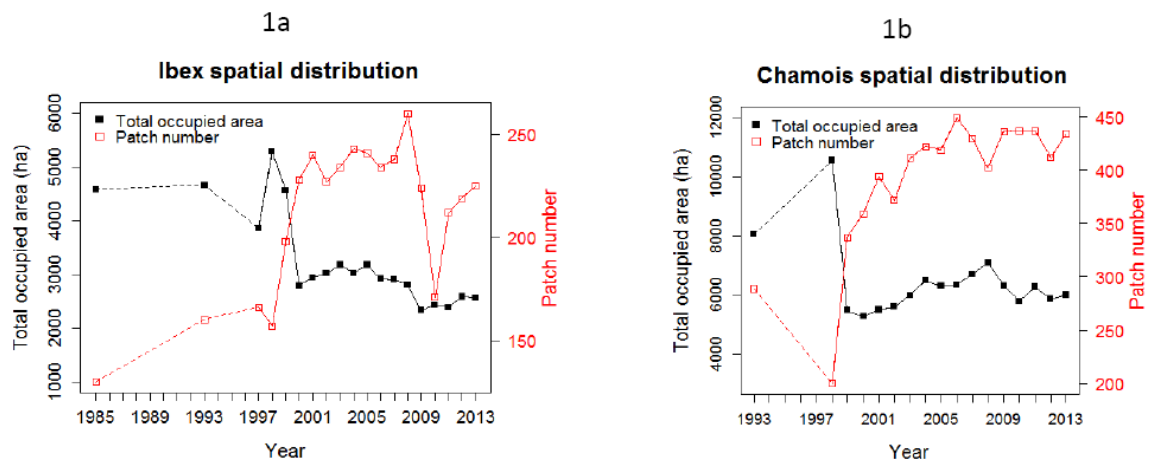

Fig.1. Trend of total area occupied by ibex (1a) and chamois (1b) and relative occupied patch number from 1985 to 2013.

When we analysed trend of ibex distribution pattern during 1999-2013 (period in which data localisation were more accurate) we observed a decrease of total occupied area (TA) (Man-Kendall Test, tau=-0.54, P<0.01) (fig.1a), less evident was the relative mean patch area decrease (Man Kendall Test, tau=-0.41, P<0.05) (Appendix table 2A).All other spatial indexes (patch number, radius of gyration, Euclidean nearest neighbour, and patch cohesion index) did not indicate any significant trend in ibex distribution pattern.

The same analysis with chamois census data pointed out a positive trend of occupied patches number (Man Kendall Test, tau=0.57, P<0.01) (fig. 1b), otherwise all other indexes showed not significant trend (Appendix table 3A).

We attached tables of all spatial indexes values for both ungulates in Appendix (table 2A-3A).

When we analysed occurrence frequency grid used by ibex (Fig.2) and chamois (Fig.3), considering September census data localisations from 2000-2013, a different distribution pattern is marked in some surveillance zones. This type of output is interesting from a management point of view, seen reduction in ibex presence area and an increase in patch number occupied by chamois.

4

Topographic and morphological characteristics of grids occupied by ibex during September census are shown in fig.4. A significant change in annual ibex elevation distribution was evident (Kruskal-Wallis chi-squared = 208.64, df = 13, P<0.001) from 2000 to 2013, otherwise considering all other environmental variables (slope, roughness and aspect) ibex annual distribution didn't change (Kruskal-Wallis Test, df = 13, P>0.05).

A slightly positive trend of mean annual elevation used by ibex was registered (Man Kendall Test, tau= 0.41, P<0.05) during 2000-2013 period (Fig.5).

Ibex occupied grids with a mean slope of 34.4°± 0.1 ES (9.3 SD) and mean roughness of 19.2± 0.1 ES (7.0 SD) in September census observations.
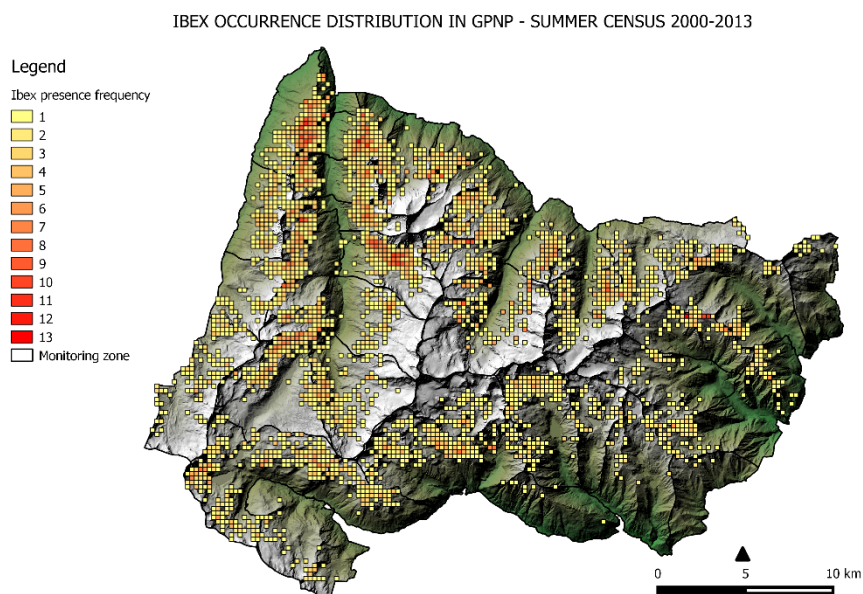

Fig. 2. Ibex occurrence distribution in Gran Paradiso National Park, considering September data census from 2000-2013.
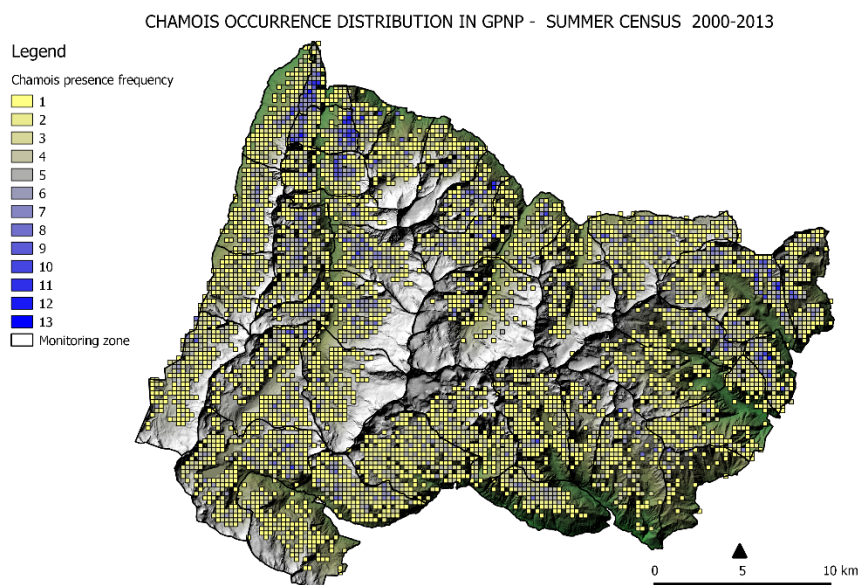
Fig. 3. Chamois occurrence distribution in Gran Paradiso National Park, considering September data census from 2000-2013.
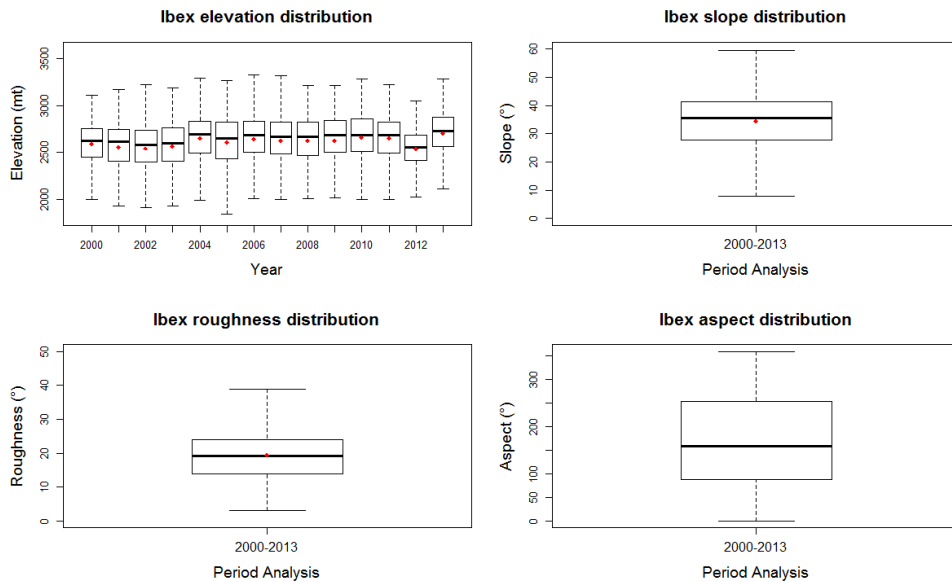

Fig. 4. Boxplots of Ibex distribution characteristics (elevation, slope, roughness and aspect) considering September census data from 2000 to 2013. Horizontal bars represent the median; red point the means; box heights the interquartile range, and whiskers span 1.5 x interquartile range. Outliers (>1.5 x interquartile range) are not shown.
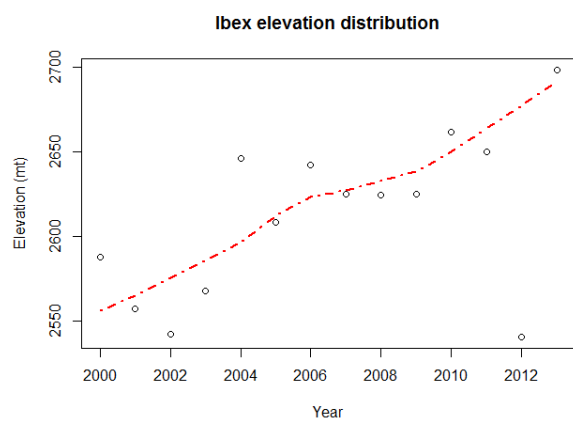

Fig. 5. Trend of annual mean elevation distribution throughout the years and relative LOWESS regression curve is represented as a dashed line (September census data from 2000 to 2013).

Graphics in Fig. 6 display topographic and morphological characteristics of grids occupied by chamois during 2000-2013 census. Chamois elevation distribution changed annually (Kruskal-Wallis chi-squared = 59.048, df = 13, P<0.001), and on the contrary any change in aspect distribution was evident (Kruskal-Wallis Test, df = 13, P >0.05). Levene test showed a different data samples variance of chamois slope (Levene-type test, Test Statistic =42.61, P < 0.001) and roughness distribution (Levene-type test, Test Statistic = 30.90, P < 0.01) throughout the years. Annual mean elevation of grids occupied by chamois didn't' showed any evident trend during 2000-2013 period (Man Kendall Test, tau= 0.08, P>0.05).
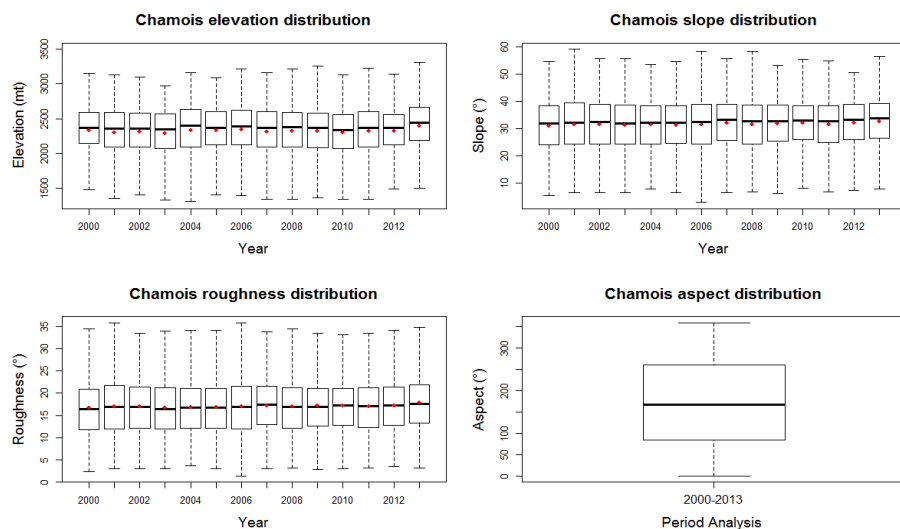

Fig. 6. Boxplots of chamois distribution characteristics (elevation, slope, roughness and aspect) considering September census data from 2000 to 2013. Horizontal bars represent the median; red point the means; box heights the interquartile range, and whiskers span 1.5x interquartile range. Outliers (>1.5 x interquartile range) are not shown.

A preliminary and descriptive analysis of ibex presence signs collected during winter season (fig. 7) pointed out an evident difference of annual variance in ibex elevation (Levene type test, Test Statistic =15.31, P < 0.01) and in ibex aspect distribution (Levene-type test, Test Statistic = 34.12, P < 0.001).
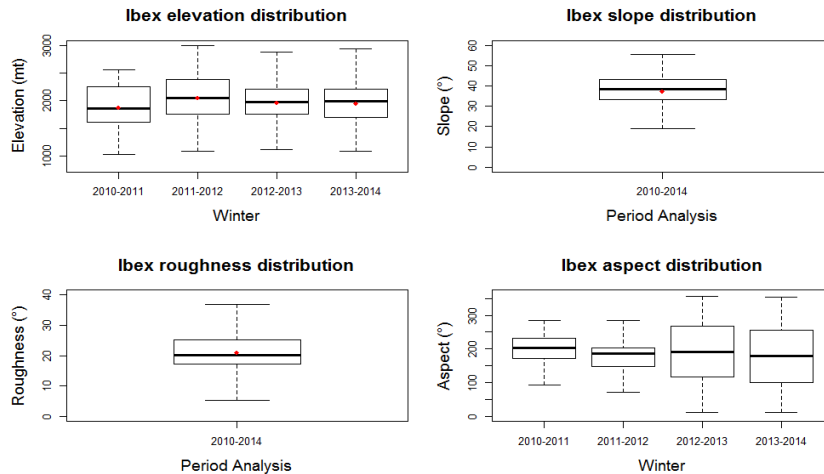
7

Fig. 7. Boxplots of ibex distribution characteristics (elevation, slope, roughness and aspect) considering occasional sign of presence (direct and indirect) collecting in winter season from 2010 to 2014. Horizontal bars represent the median; red point the means; box heights the interquartile range, and whiskers span 1.5x interquartile range. Outliers (>1.5 x interquartile range) are not shown.

Otherwise, ibex did not change its slope (Kruskal-Wallis Test, df = 13, P >0.05) and roughness distribution throughout years (Kruskal-Wallis Test, df = 13, P >0.05).

Ibex occupied grids with a mean slope of 37.3°±0.3 ES (8.9 SD), and mean roughness of 21.0± 0.2 ES (6.8 SD) in winter season.

When we analysed the same type of data in chamois winter distribution (Fig. 8) we found a significant change in annual elevation use (Kruskal-Wallis chi-squared = 27.83, df = 3, P<0.01), slope use (Kruskal-Wallis chi-squared = 33.69, df = 3, P<00.1) and roughness use (Kruskal-Wallis chi-squared = 33.25, df = 3, P<0.01).

A significant difference was evident in annual variance of data sample in chamois aspect distribution during winter season (Levene-type test, Test Statistic = 18.69, P< 0.01).
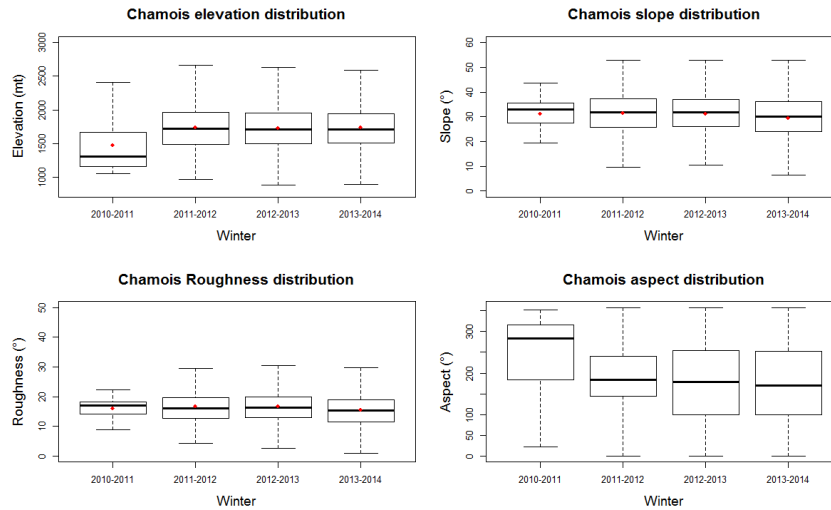
Fig. 8. Boxplots of chamois distribution characteristics (elevation, slope, roughness and aspect) considering occasional sign of presence (direct and indirect) collecting in winter season from 2010 to 2014. Horizontal bars represent the median; red point the means; box heights the interquartile range, and whiskers span 1.5 x interquartile range. Outliers (>1.5 x interquartile range) are not shown.

Roe deer signs of presence showed a significant annual change in elevation specie distribution during winter (Kruskal-Wallis chi-squared = 13.37, df = 3, P<0.01) and spring-summer season (Kruskal-Wallis chi-squared = 24.55, df = 2, P<0.01) and also regarding aspect distribution (Kruskal-Wallis chi-squared = 20.96, df = 2, P<0.01).

Levene test showed a different annual variance of data sample in roe deer aspect distribution during winter (Levene-type test, Test Statistic = 31.04, P<0.01). Descriptive graphics of environmental features of grid used by roe deer are in Fig.9.
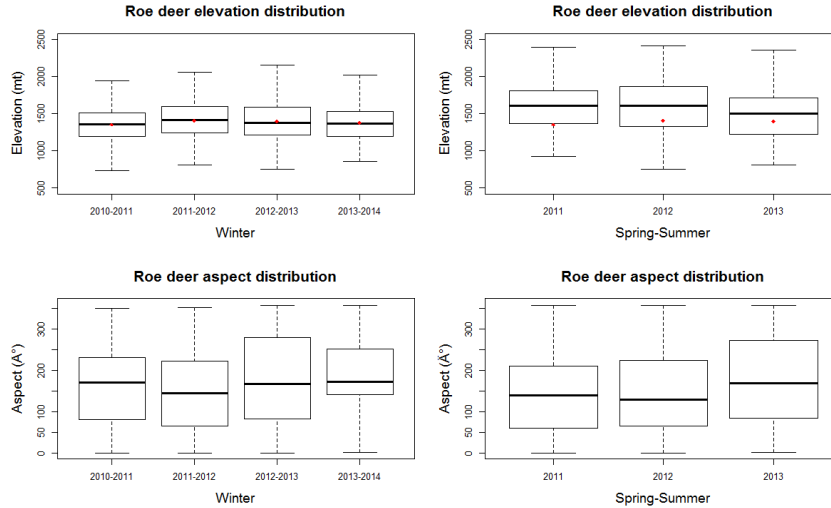
Fig. 9. Boxplots of roe deer distribution characteristics (elevation, slope, roughness and aspect) considering occasional sign of presence (direct and indirect)   collecting in winter season from 2010 to 2014. Horizontal bars represent the median; red point the means; box heights the interquartile range, and whiskers span 1.5 x interquartile range. Outliers (>1.5 x interquartile range) are not shown.

When we evaluated ibex and chamois pattern distribution from data census, we pointed out around an intermediate range of value in environmental feature grids (elevation, slope e roughness) where both ungulates (C-I) have been observed during 2000-2013 period (spatial overlap) compared to grids where unique specie observations were done (C, I) (Fig. 10).
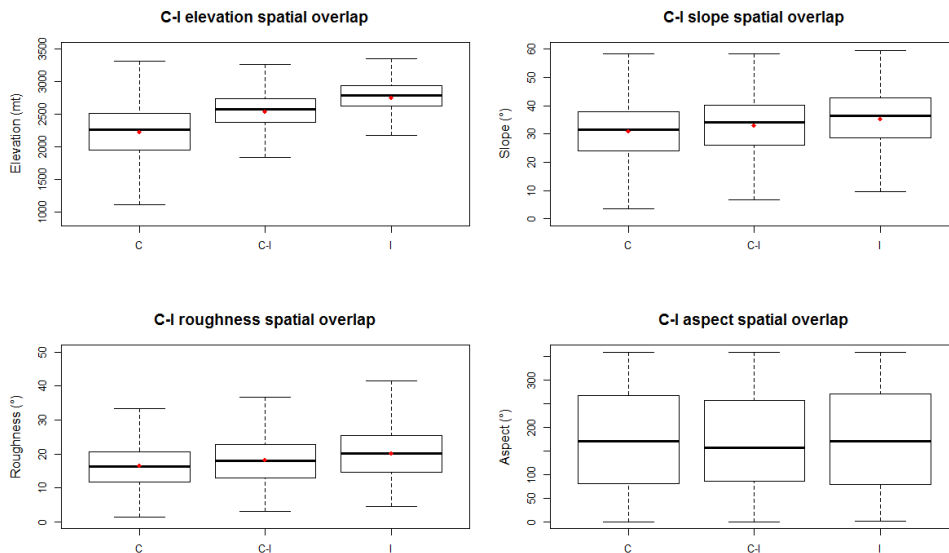
Fig. 10. Boxplots of Chamois (C) and Ibex (I) distribution characteristics (elevation, slope, roughness and aspect) considering overlap grids, where both ungulate had been observed (C-I), and grids where only one species had been observed (C or I) from 2000-2013 census data. Horizontal bars represent the median; red point the means; box heights the interquartile range, and whiskers span 1.5x interquartile range. Outliers (>1.5 x interquartile range) are not shown.

A non-conformity variance between groups (C=Chamois presence grids, C-I= Chamois-ibex presence grids, I=ibex presence grids) has been underlined respect to elevation (Levene-type test, Test Statistic = 381.99, P<0.01), roughness (Levene-type test, Test Statistic = 41.06, P<0.01) and aspect (Levene-type test, Test Statistic =34.78, P<0.01) distribution. A significant difference in slope distribution among groups has been pointed out (Kruskal-Wallis chi-squared = 167.60, df = 2, P<0.001).

It seems that chamois distribution pattern have an expansive range considering topographic and morphological features of occupied grids, especially elevation. The mean elevation of grids, where only chamois (C) had been observed, was 2225 ± 7 ES mt (398 SD); chamois and ibex (C-I) was 2538 ± 7 ES mt (293 SD) e only ibex (I) was mt 2759 ± 9 ES mt (265 SD).The mean slope of grids, where only chamois (C) had been observed, was 30.9° ± 1.2 ES (9.3 SD), chamois and ibex (C-I) was 33.0° ± 1.4 (9.6 SD), and only ibex was 35.3°± 1.7 ES (9.4 SD). The mean roughness of grids, where only chamois (C) had been observed, was 16.5 ± 0.8 ES (6.3 SD), chamois and ibex (C-I) was 18.3 ± 1.0 ES (7.0 SD), and only ibex was 20.2± 1.3 ES (7.3 SD).
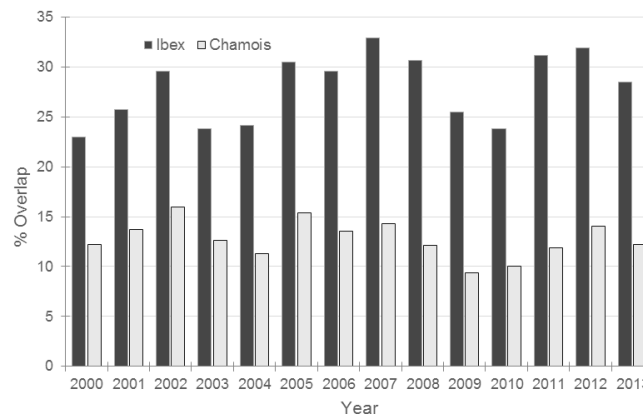

Fig.11. Barplots of Chamois and Ibex overlap percentage considering area, where both ungulates had been observed during census from 2000-2013, relative to the total area occupied by each species every year.

We analysed the annual spatial overlap (percentage) between chamois and ibex, considering the area of grids where both alpine ungulate had been observed during census, respect to total grid area occupied by species (Fig.11). Spatial overlap percentage in ibex annual distribution pattern had a range between 23.0 (2000) and 32.9% (2007), otherwise chamois had a range between 9.4 (2009) e 16.0% (2002). No evident linear trend in spatial overlap for both alpine ungulates was pointed out during 2000-2013 period.

11

Discussion

Monitoring ungulate distribution especially for protected species (as alpine ibex) is fundamental for management and conservation aim in a protected area as Gran Paradiso National Park.

Long-term census data are extremely valuable for the analysis of ungulate spatial use patterns and relative trends of spatial indexes (like those of landscape ecology) could investigate change in spatial distribution pattern, if methodology remains the same over the years.

Thanks to monitoring data and their preliminary analysis we could identify particular set of problems (in ungulate abundance, dynamic or distribution), which could be analysed with detailed research and protocols with specific topics in appropriate study area.

In literature, many studies report factors that could influence animal distribution. These factors could be: hunting, predation or species competition (Focardi et al. 2006; Festa-Bianchet & Apollonio 2003); changes in landscape composition (Beauchesne et al. 2013; Li et al. 2013; Wiens 1976); climatic changes (Walther et.al. 2002; Herfindal et al. 2012), finally anthropogenic activity (Schroeder et al. 2014; Bagchi et al. 2004, Mason et al. 2014).

Climatic change, also, affects habitat quality and food availability (Tveraa et al. 2013; Pettorelli & Pelletier 2007) and these effects probably influences annual ungulate distribution too. Spatial indexes analysis pointed out a decrease in total occupied area by ibex and relative mean patch area considering 2000-2013 period, when data localisation are more accurate. In landscape ecology, scale of resolution has a fundamental importance, because there are different effects of process in a landscape considering different scales of analysis (Turner et al. 1989). In Zurlo (2014), the analysis at different scales (250 m grid and 500 m grid) confirm an effective change in alpine ibex distribution pattern. This preliminary study pointed out also a no significant correlation between decrease in ibex abundance from census data and occupied area by the specie (Zurlo 2014).

On the contrary, considering chamois census data, the same analysis pointed out an increase in number of patches, but no trend in total occupied area by the specie was evident. Chamois distribution seem to be more outspread during the last years without significant changing in total occupied area.

Future research focus on this topic are necessary to assess factors and their effects on ibex and chamois distribution and their possible interaction.

When considering the environmental characteristic of spatial use from census data, both ibex and chamois elevation distribution pointed out that the use changed during 2000-2013 period.

Alpine ungulate distribution could be conditioned by weather condition of the census days and by seasonal climate condition, which could influence phenological development and so trophic seasonal resource geographical range. An increasing trend in ibex elevation distribution had been observed from 2000 to 2013 from summer census.

Those preliminary analysis results from census data (slight increasing trend in elevation ibex distribution, decreasing total area occupied by ibex, and chamois outspread) have to be investigate with a more specific analysis to focus possible effect of climate change in alpine ungulate distribution.

In Swiss a research had pointed out a relevant increase on mean elevation distribution (250 m) of male ibex, during a period of 19 years (Herfindal et al. 2012). This event could be an indicator of climatic warming in alpine ecosysteme. Predictive models that could appreciate ungulate distribution under future scenario is a goal of action 3.b.

Ibex had a typical alpine distribution considering topographic characteristics in space use, while chamois was less selective and range of distribution are more expansive and variable, especially in elevation distribution concurring to specie ecological knowledge.

Spatial overlap between two alpine species occurred in a halfway range for the most of environmental characteristics analysed and considering percentage of overlap every year it was more important in ibex distribution (23.0-32.9 %).

An occupancy model, structured with a robust study design, is necessary to examine species interaction in space use and possible trophic resource competition between alpine ungulates (MacKenzie et al. 2006), topic of student Master thesis in Levionaz study area (Silba 2014). It seems that chamois presence had a negative effect on ibex distribution and a temporal partition of space and trophic resource use between two species have been supposed (Silba 2014).

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APPENDIX

Methods

Table 1A. Fragstats composition metrics and spatial configuration metrics.

AREA	
$Area = a_{ij}\left(\dfrac{1}{10000}\right)$	a_{ij} =area (m²) of patch ij
	Hectares
Description	AREA equals the area (m²) of the patch, divided by 10,000 (to convert to hectares)
Range	AREA > 0, without limit.

NUMBER OF PATCHES	
$NP = n_i$	n_i = number of patches in landscape
	No units
Description	NP equals the number of patches
Range	NP ≥ 1, without limit. NP= 1, patch corresponds to landscape

RADIUS OF GYRATION	
$Gyrate = \sum\limits_{r=1}^{z} \dfrac{h_{ijr}}{z}$	h_{ijr} = distance (m) between cell ijr, located within patch ij, and the centroid of patch ij based on cell center-to-cell center distance z = number of cells in patch ij
	Meters
Description	GYRATE equals the mean distance (m) between each cell in the patch and the patch centroid
Range	GYRATE ≥ 0, without limit GYRATE = 0 when the patch consists of a single cell and increases without limit as the patch increases in extent. GYRATE achieves its maximum value when the patch comprises the entire landscape

EUCLIDEAN NEAREST NEIGHBOR	
$ENN = h_{ij}$	h_{ij} = distance (m) from patch ij to nearest neighboring patch of the ij same type (class), based on patch edge-to-edge distance, computed from cell center to cell center
	Meters
Description	ENN equals the distance (m) to the nearest neighboring patch of the same type, based on shortest edge-to-edge distance.
Range	ENN > 0, without limit ENN approaches 0 as the distance to the nearest neighbor decreases. The minimum ENN is constrained by the cell size while the upper limit is constrained by the extent of the landscape

PATCH COHESION INDEX	
$Cohesion = \left[1 - \dfrac{\sum_{j=1}^{n} p_{ij}^*}{\sum_{j=1}^{n} p_{ij}^* \sqrt{a_{ij}^*}}\right] \cdot \left[1 - \dfrac{1}{\sqrt{Z}}\right]^{-1} \cdot (100)$	p_{ij}^*= perimeter of patch ij in terms of number of cell surfaces a_{ij}^* = area of patch ij in terms of number of cells Z= total number of cells in the landscape
	No units
Description	COHESION equals 1 minus the sum of patch perimeter divided by the sum of patch

		perimeter times the square root of patch area for patches, divided by 1 minus 1 over the square root of the total number of cells in the landscape, multiplied by 100 to convert to a percentage										
Range		0 < COHESION < 100 Cohesion approaches to 0 if patches become increasingly subdivided and less physically connected; while its value increases if proportion of the landscape comprised in patches increase										

Results

TA= Total Area occupied; NP= Number of patches; PA=Patch area; RG= Radius of gyration; ENN= Euclidean nearest neighbour; PCI=patch cohesion index; SD=standard deviation; CV= Coefficient of variation

ANNO	TA	NP	PA			RG			ENN			PCI
			MEAN	SD	CV	MEAN	SD	CV	MEAN	SD	CV	
1985	4587.50	130	35.29	35.03	99.27	241.70	145.23	60.09	755.08	530.37	70.24	67.47
1993	4668.75	160	29.18	45.24	155.04	215.72	170.30	78.94	751.21	331.51	44.13	68.6
1997	3868.69	166	23.31	25.63	109.96	193.34	109.83	56.81	819.05	557.39	68.05	59.71
1998	5287.42	157	33.68	51.14	151.86	232.08	194.45	83.79	711.37	299.14	42.05	71.23
1999	4575.00	198	23.11	26.96	116.67	193.05	117.54	60.89	750.91	441.92	58.85	60.20
2000	2800.00	228	12.28	11.33	92.29	154.66	70.62	45.66	694.97	337.72	48.59	40.34
2001	2943.75	240	12.27	11.01	89.76	154.75	61.24	39.57	702.65	268.15	38.16	40.42
2002	3018.75	227	13.30	18.46	138.85	157.95	90.77	57.46	699.68	304.58	43.53	50.60
2003	3181.25	234	13.60	19.14	140.76	162.69	84.31	51.82	694.28	301.48	43.42	48.33
2004	3025.00	243	12.45	15.58	125.15	155.43	79.62	51.22	702.42	342.73	48.79	45.91
2005	3181.25	241	13.20	18.75	142.08	156.21	87.67	56.12	730.68	396.21	54.23	49.75
2006	2918.75	234	12.47	16.38	131.31	156.84	84.68	53.99	739.67	349.99	47.32	45.61
2007	2906.25	238	12.21	16.14	132.15	152.44	71.42	46.85	747.16	309.69	41.45	44.88
2008	2812.50	260	10.82	9.42	87.12	149.93	62.95	41.99	689.69	295.49	42.84	35.82
2009	2331.25	224	10.41	11.80	113.34	144.18	56.24	39.01	729.4	328.7	45.06	35.67
2010	2437.46	171	14.25	9.85	69.09	158.93	60.48	38.05	863.76	471.28	54.56	41.97
2011	2387.46	212	11.26	9.08	80.65	150.56	58.29	38.72	790.99	460.3	58.19	37.02
2012	2587.46	219	11.81	12.58	106.48	155.77	77.53	49.77	731.16	345.69	47.28	42.21
2013	2568.71	225	11.42	14.14	123.89	151.31	72.94	48.21	751.11	434.28	57.82	43.91

Table 2A. Composition and spatial configuration metric of ibex spatial distribution.

TA= Total Area occupied; NP= Number of patches; PA=Patch area; RG= Radius of gyration; ENN= Euclidean nearest neighbour; PCI=patch cohesion index; SD=standard deviation; CV= Coefficient of variation

ANNO	TA	NP	PA			RG			ENN			PCI
			MEAN	SD	CV	MEAN	SD	CV	MEAN	SD	CV	
1993	8056.12	289	27.88	38.27	137.27	213.01	140.54	65.98	632.52	241.64	38.20	66.31
1998	10562.33	200	52.81	83.02	157.21	289.04	225.73	78.10	641.73	227.17	35.40	77.48
1999	5487.41	337	16.28	15.63	95.96	169.23	81.96	48.43	676.49	265.59	39.26	49.06
2000	5281.17	359	14.71	19.49	132.47	164.73	94.90	57.61	654.35	230.25	35.19	50.83
2001	5506.16	394	13.98	15.16	108.50	162.36	83.98	51.72	623.21	195.75	31.41	47.06
2002	5599.91	372	15.05	23.07	153.24	167.10	103.34	61.84	638.03	210.21	32.95	54.90
2003	5999.90	411	14.60	21.35	146.24	165.64	100.41	60.62	630.35	214.31	34.00	53.60
2004	6493.65	422	15.39	19.65	127.72	170.66	102.37	59.99	612.02	182.08	29.75	51.25
2005	6306.15	419	15.05	19.42	129.03	170.47	102.01	59.84	603.34	172.54	28.60	51.60
2006	6356.15	449	14.16	16.61	117.34	167.65	92.70	55.29	598.64	161.96	27.05	48.92
2007	6706.14	429	15.63	19.08	122.04	169.10	95.90	56.71	609.91	181.70	29.79	52.06
2008	7093.64	402	17.65	27.01	153.07	182.45	117.45	64.37	601.34	150.72	25.06	58.51
2009	6324.90	436	14.51	21.35	147.16	166.67	102.82	61.69	610.46	158.51	25.97	52.74
2010	5793.66	437	13.26	16.24	122.49	161.53	88.01	54.48	622.15	191.93	30.85	47.20
2011	6274.90	437	14.36	17.79	123.91	167.96	93.52	55.68	617.75	177.65	28.76	49.80
2012	5862.41	412	14.23	17.92	125.96	166.44	93.09	55.93	614.09	191.22	31.14	50.27

| 2013 | 6006.15 | 434 | 13.84 | 19.11 | 138.08 | 164.19 | 95.42 | 58.12 | 614.20 | 174.07 | 28.34 | 50.66 |

Table 3A. Composition and spatial configuration metric of chamois spatial distribution.

IBEX DISTRIBUTION IN GPNP - SUMMER CENSUS 1985-1992
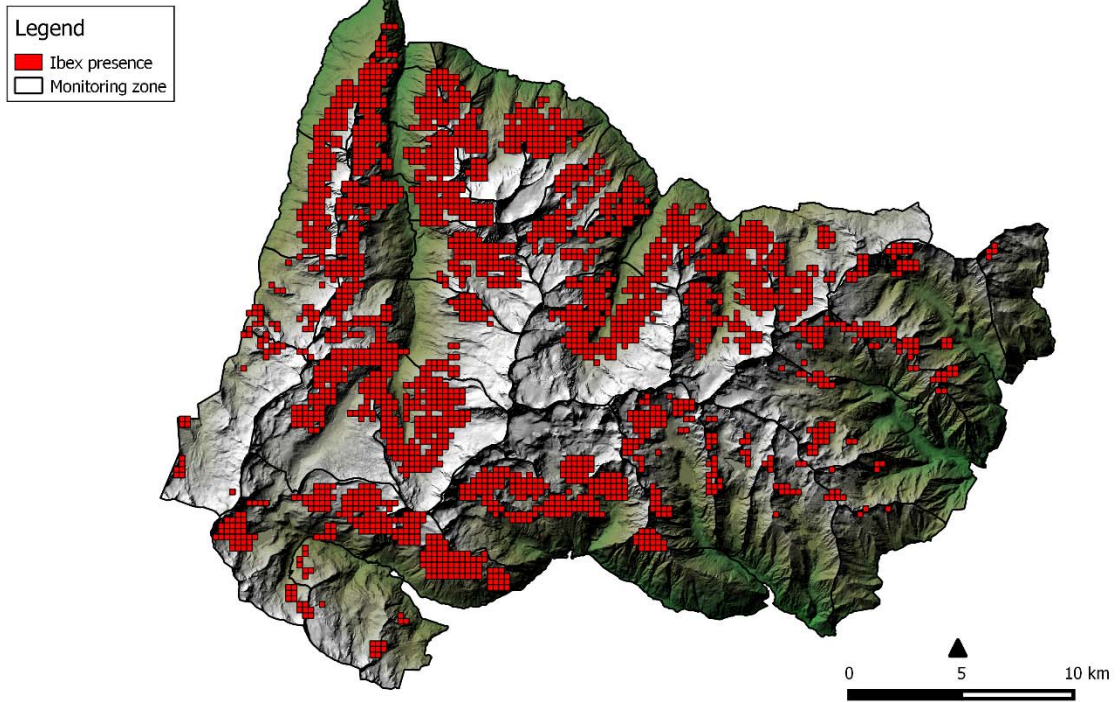

Fig. 1A. Ibex presence distribution from September census data (1985-1992).

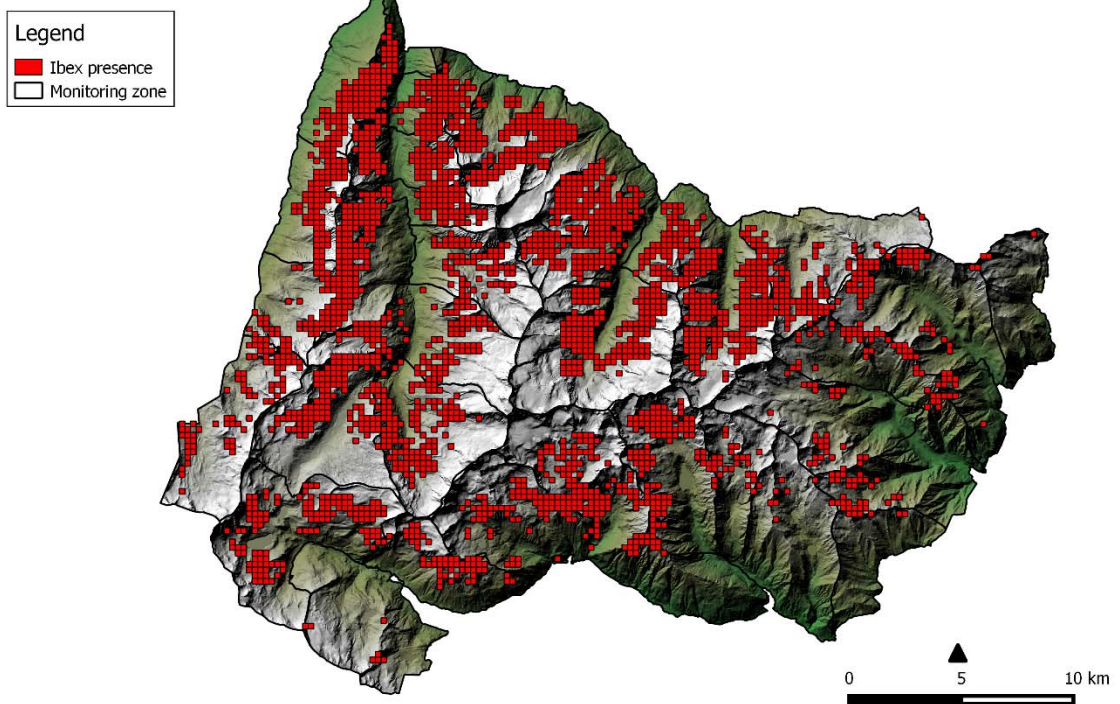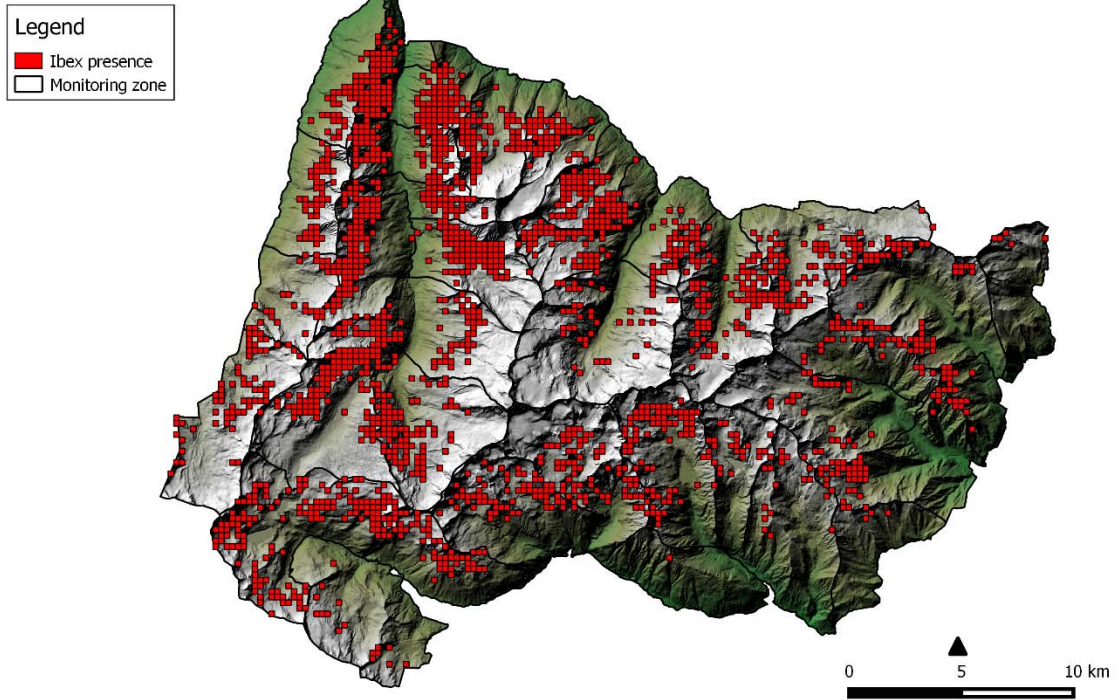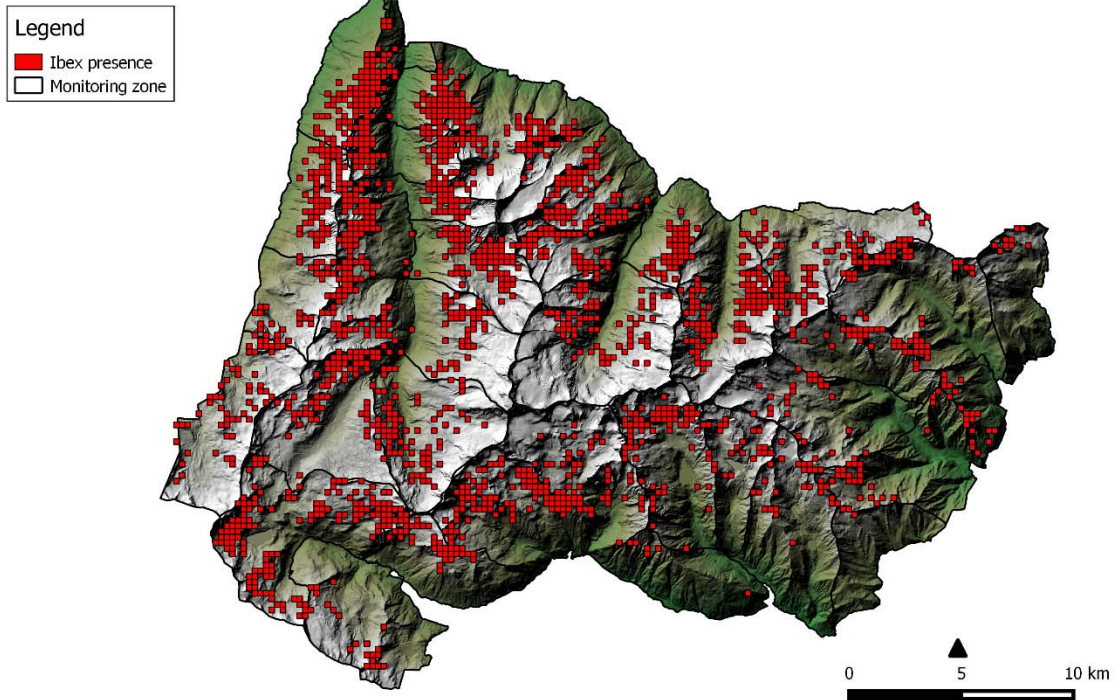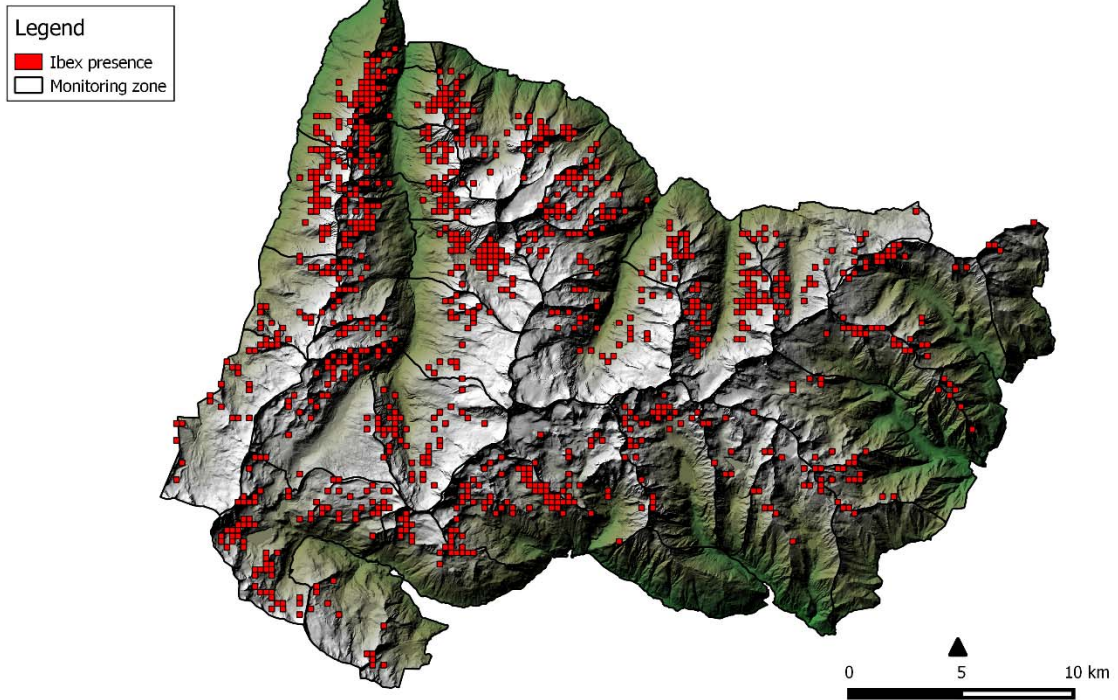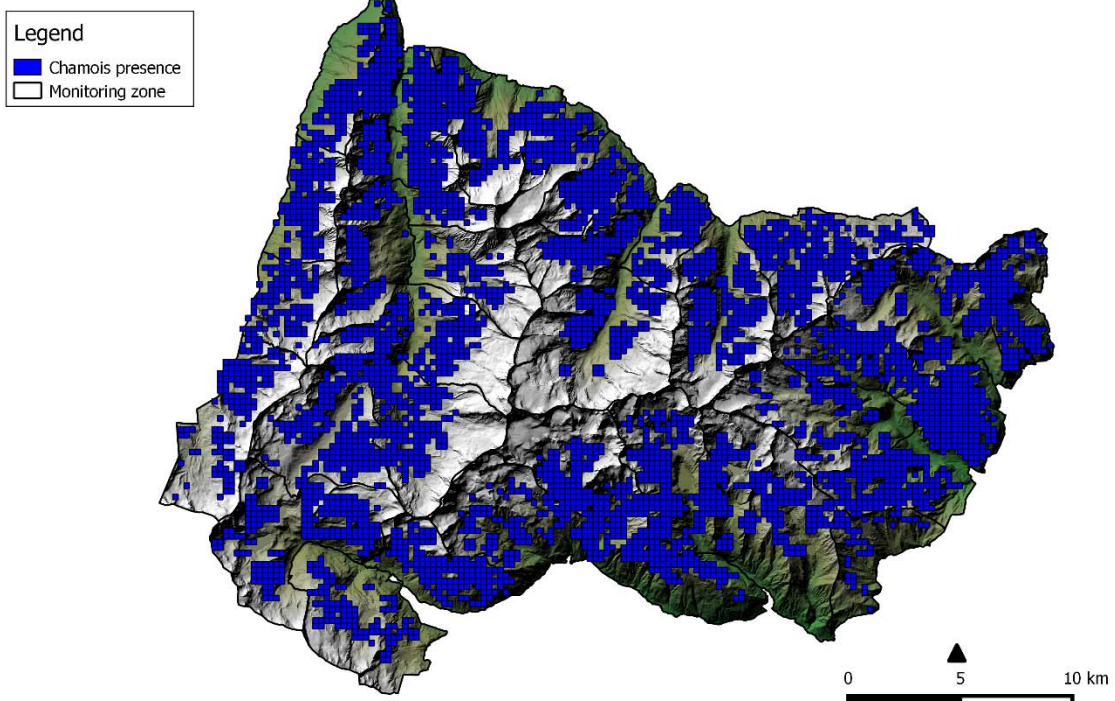IBEX DISTRIBUTION IN GPNP - SUMMER CENSUS 1993-1999

Fig. 2A. Ibex presence distribution from September census data (1993-1999).
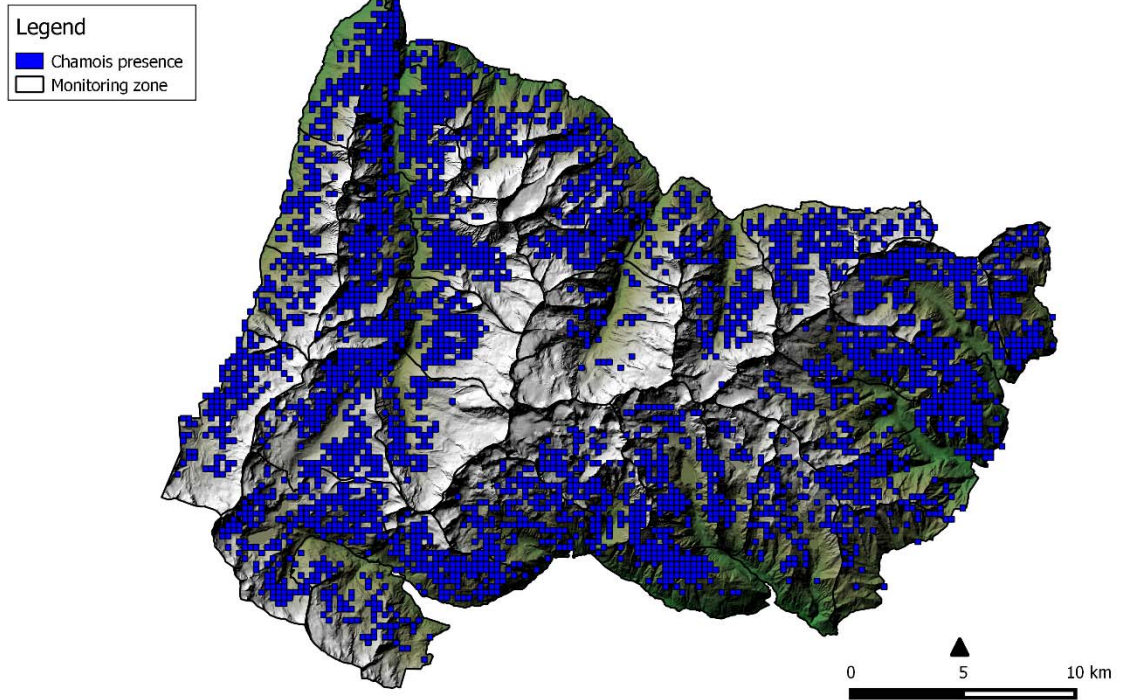
IBEX DISTRIBUTION IN GPNP - SUMMER CENSUS 2000-2006

Legend
Ibex presence
Monitoring zone

0   5   10 km

Fig. 3A. Ibex presence distribution from September census data (2000-2006).

IBEX DISTRIBUTION IN GPNP - SUMMER CENSUS 2007-2013
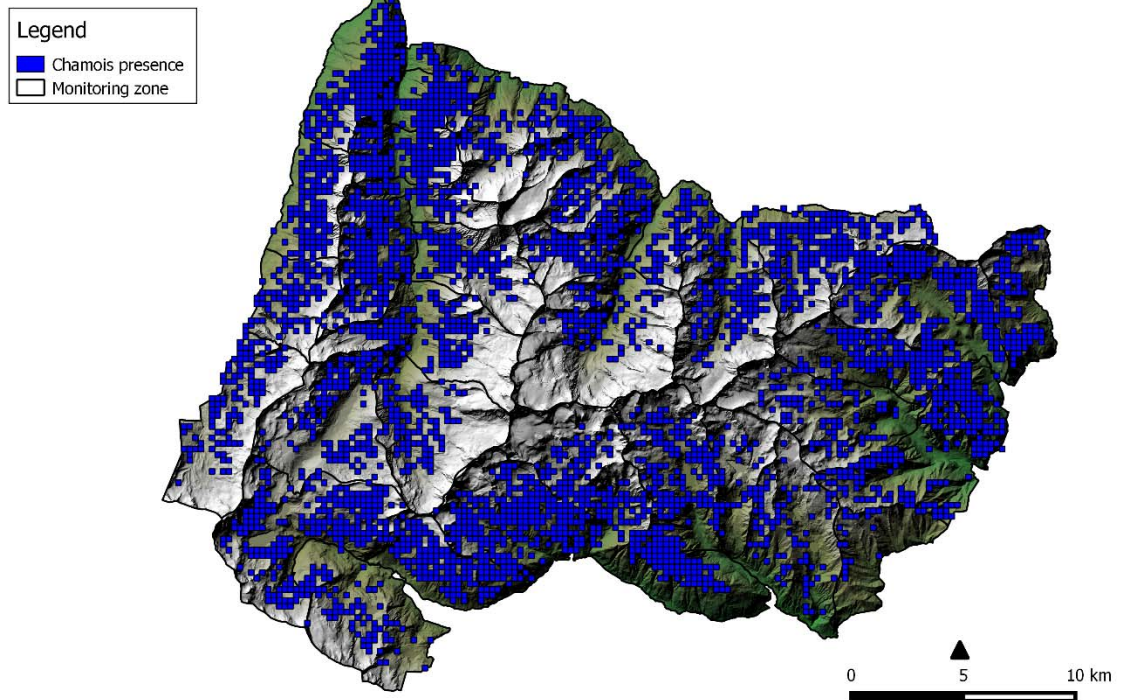
Legend
Ibex presence
Monitoring zone

0   5   10 km

Fig. 4A. Ibex presence distribution from September census data (2007-2013).

IBEX DISTRIBUTION IN GPNP - SUMMER CENSUS 2011-2013

Fig. 5A. Ibex presence distribution from September census data (2011-2013).
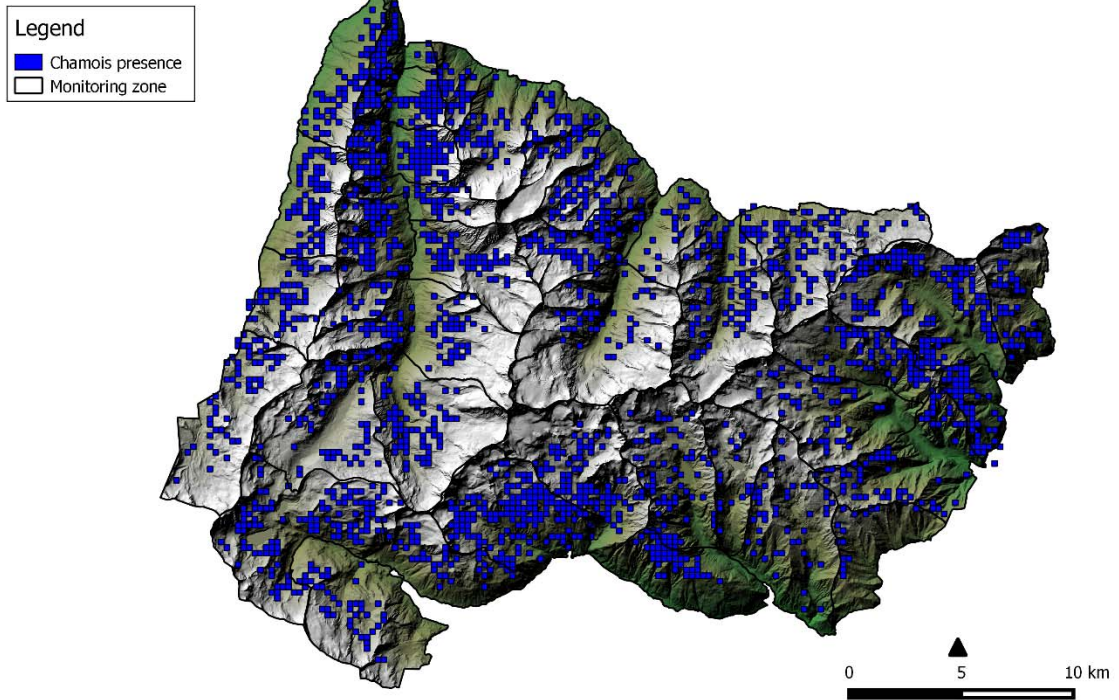
CHAMOIS DISTRIBUTION IN GPNP - SUMMER CENSUS 1993-1999

Fig. 6A. Chamois presence distribution from September census data (1993-1999).

Fig. 7A. Chamois presence distribution from September census data (2000-2006).

Fig. 8A. Chamois presence distribution from September census data (2007-2013).

CHAMOIS DISTRIBUTION IN GPNP - SUMMER CENSUS 2011-2013

Fig. 9A. Chamois presence distribution from September census data (2011-2013).

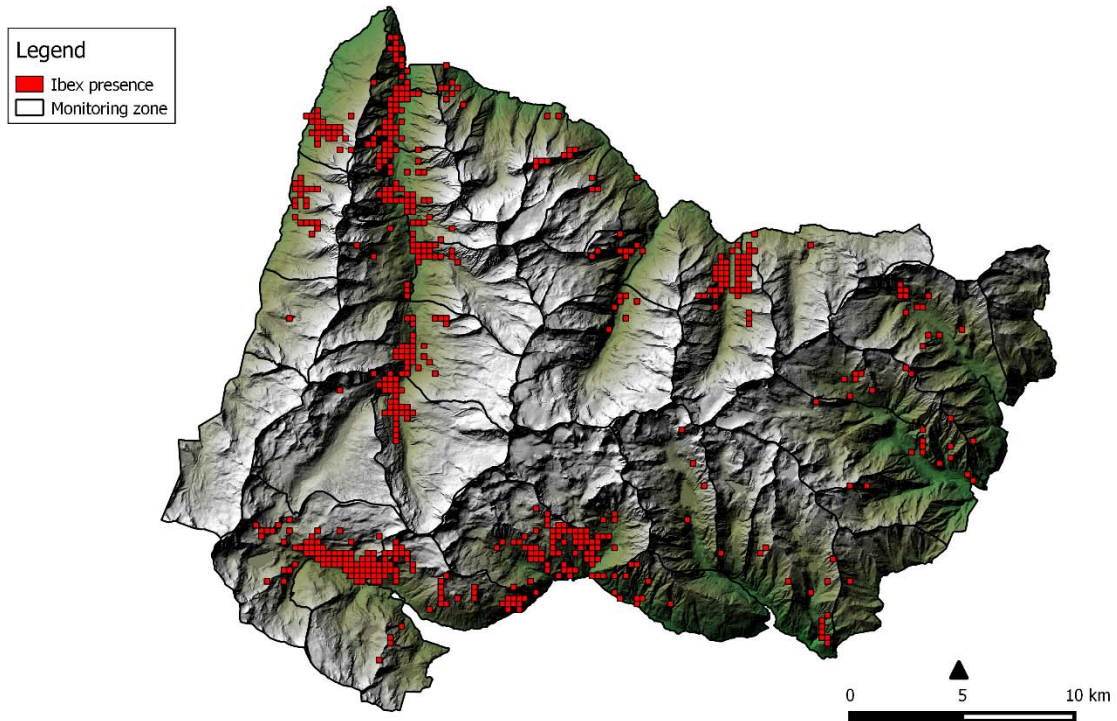IBEX DISTRIBUTION IN GPNP - WINTER OCCASIONAL OBSERVATIONS 2010-2014

Fig. 10A. Ibex presence distribution from winter occasional observations of specie presence signs (direct and indirect) (2010-2014).

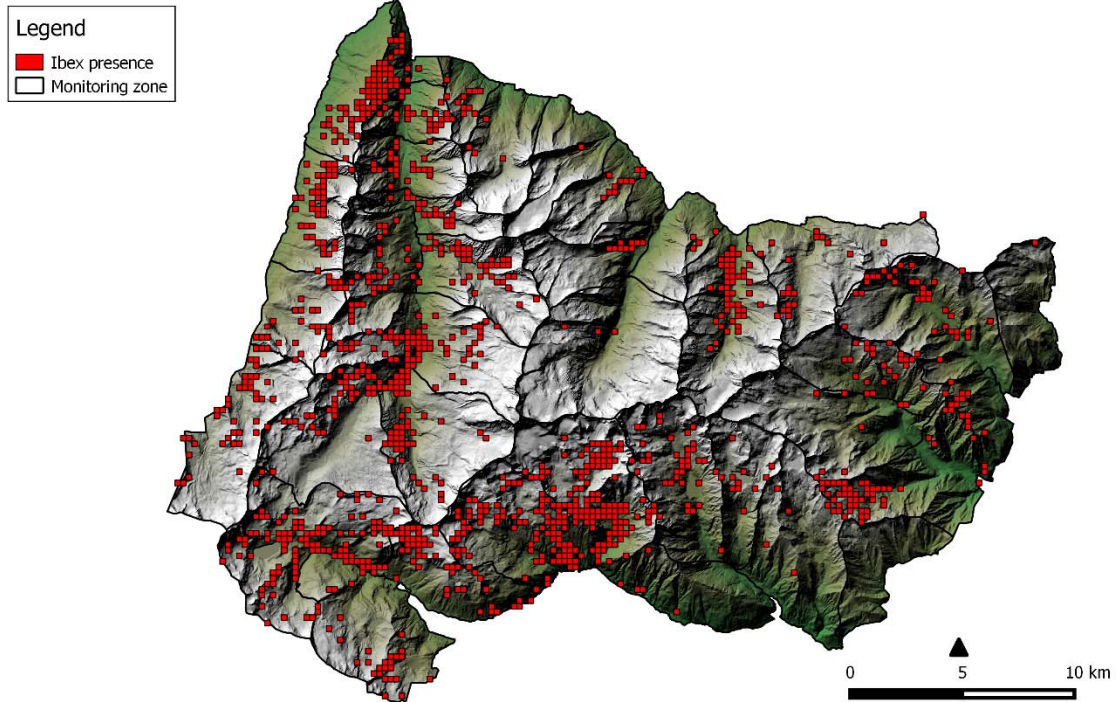IBEX DISTRIBUTION IN GPNP - SPRING-SUMMER OCCASIONAL OBSERVATIONS 2011-2013

Fig. 11A. Ibex presence distribution from spring-summer occasional observations of specie presence signs (direct and indirect) (2011-2013).

CHAMOIS DISTRIBUTION IN GPNP - WINTER OCCASIONAL OBSERVATIONS 2010-2014
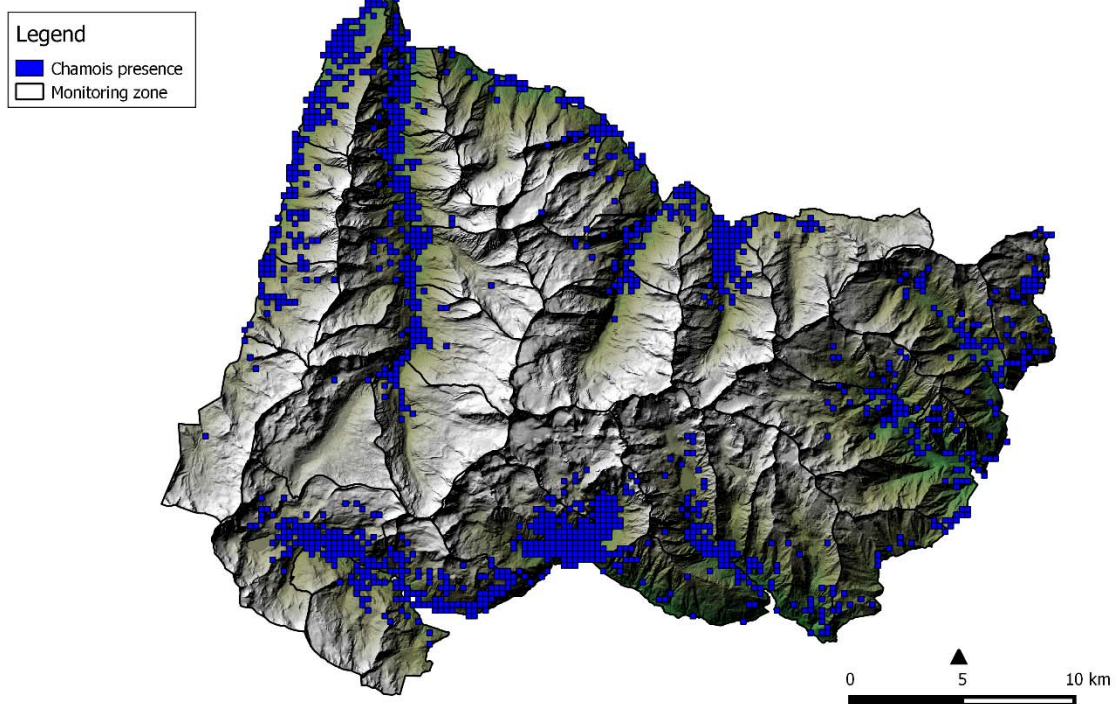

Fig. 12A. Chamois presence distribution from winter occasional observations of specie presence signs (direct and indirect) (2010-2014).

CHAMOIS DISTRIBUTION IN GPNP - SPRING-SUMMER OCCASIONAL OBSERVATIONS 2011-2013
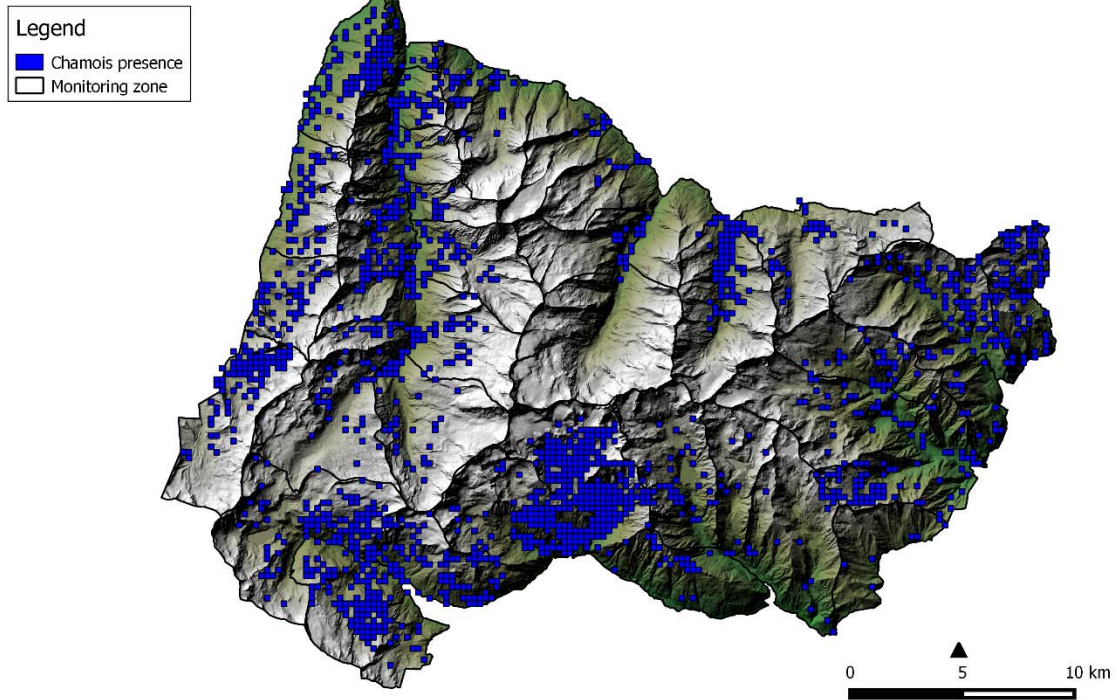

Fig. 13A. Chamois presence distribution from spring-summer occasional observations of specie presence signs (direct and indirect) (2011-2013).

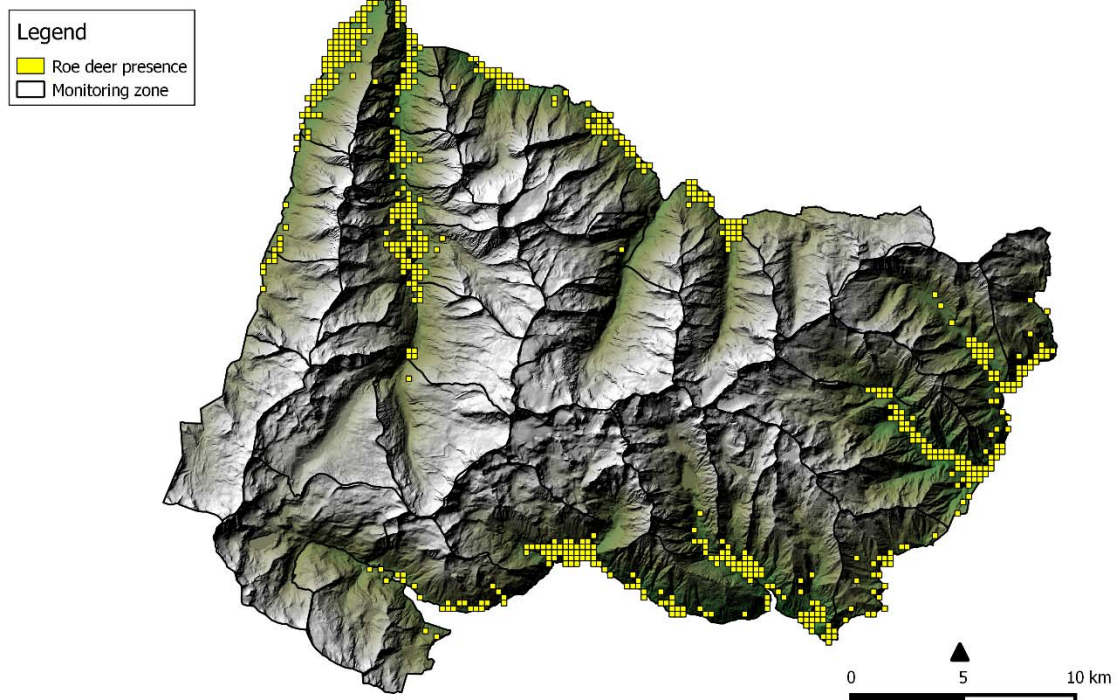ROE DEER DISTRIBUTION IN GPNP - WINTER OCCASIONAL OBSERVATIONS 2010-2014

Fig. 14A. Roe deer presence distribution from winter occasional observations of specie presence signs (direct and indirect) (2010-2014).

ROE DEER DISTRIBUTION IN GPNP - SPRING-SUMMER OCCASIONAL OBSERVATIONS 2011-2013

Legend
Roe deer presence
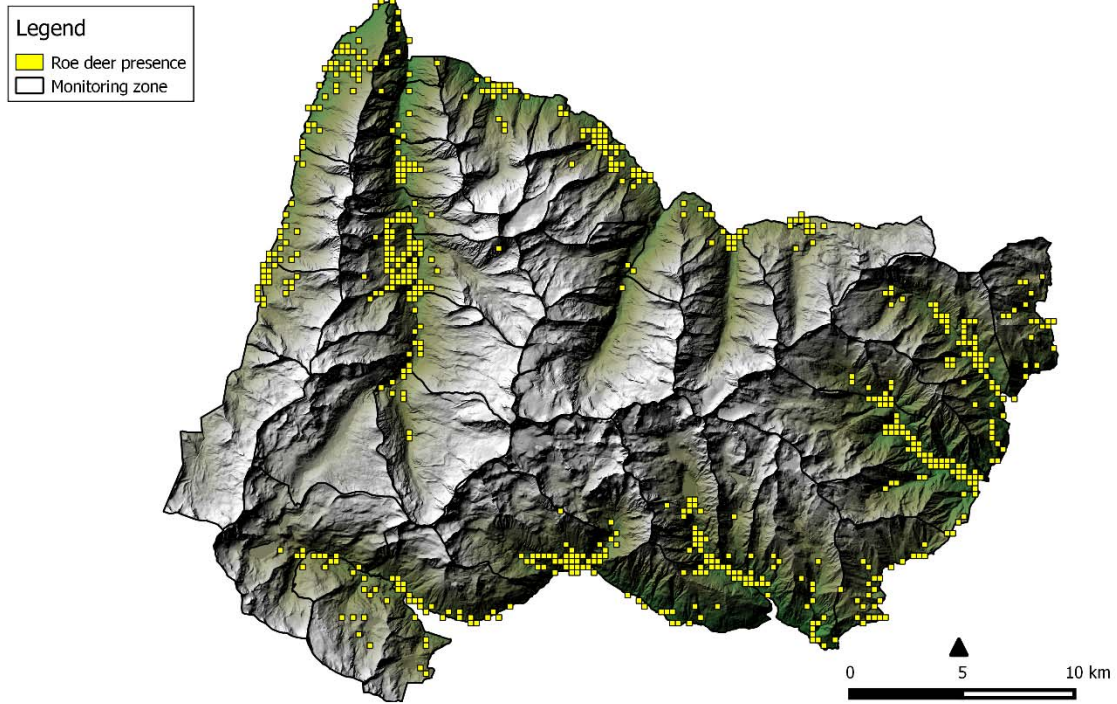Monitoring zone

0   5   10 km

Fig. 15A. Roe deer presence distribution from spring-summer occasional observations of specie presence signs (direct and indirect) (2011-2013).

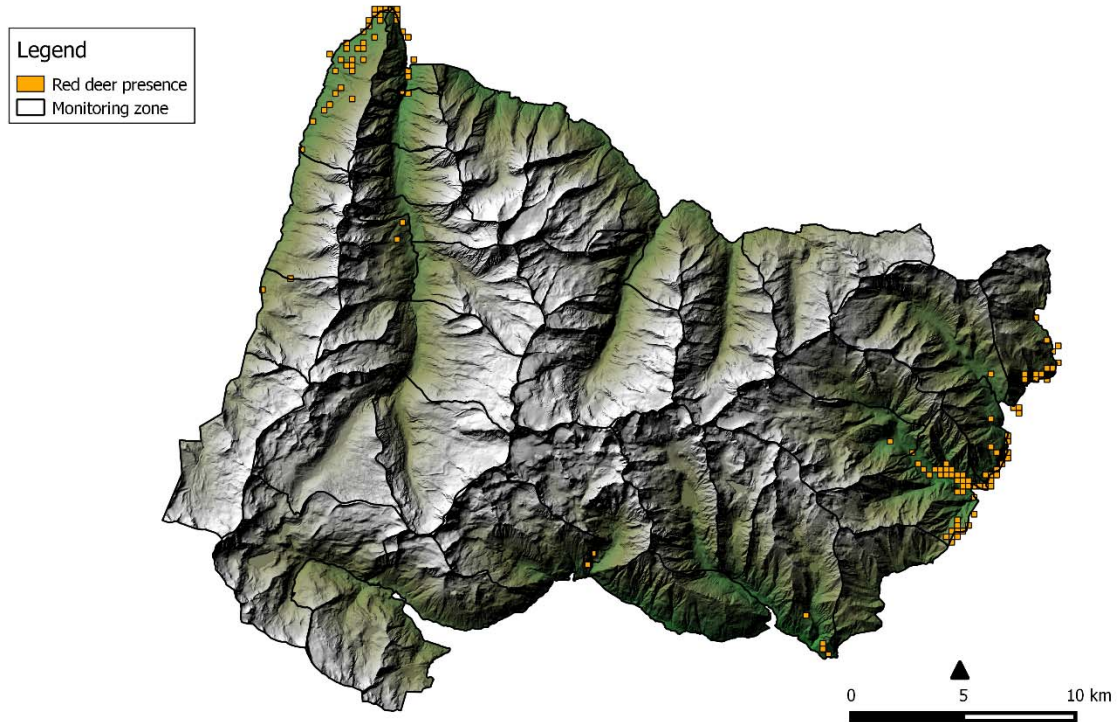RED DEER DISTRIBUTION IN GPNP - WINTER OCCASIONAL OBSERVATIONS 2010-2014

Legend
Red deer presence
Monitoring zone

0   5   10 km

Fig. 16A. Red deer presence distribution from winter occasional observations of specie presence signs (direct and indirect) (2010-2014).
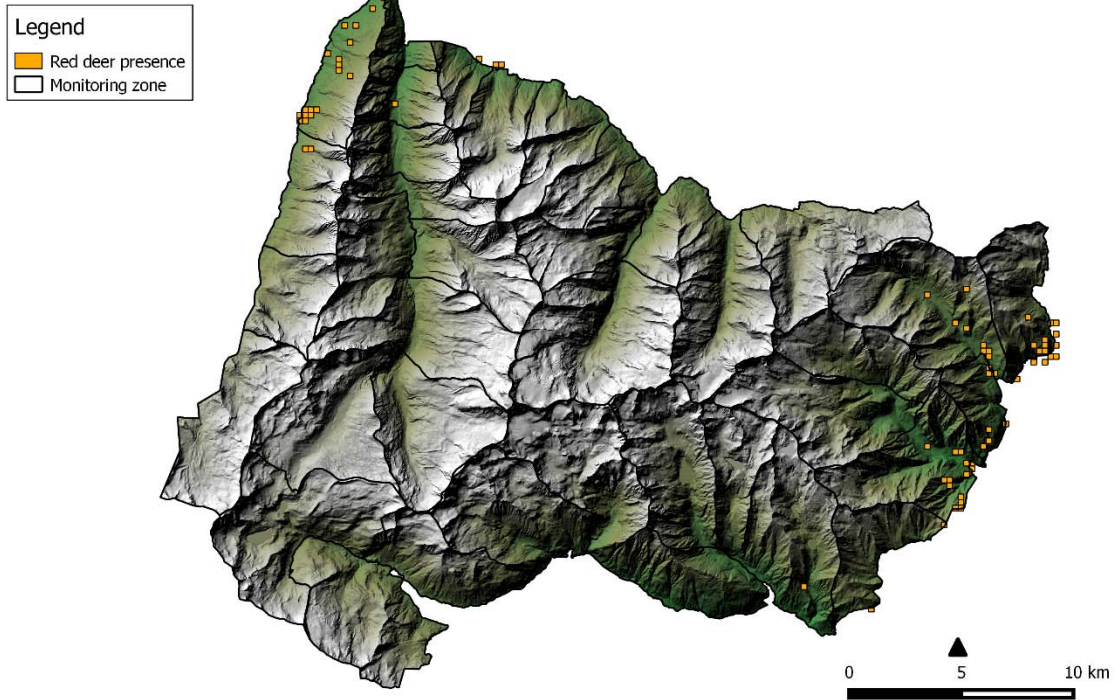
Fig. 17A. Red deer presence distribution from spring-summer occasional observations of specie presence signs (direct and indirect) (2011-2013).

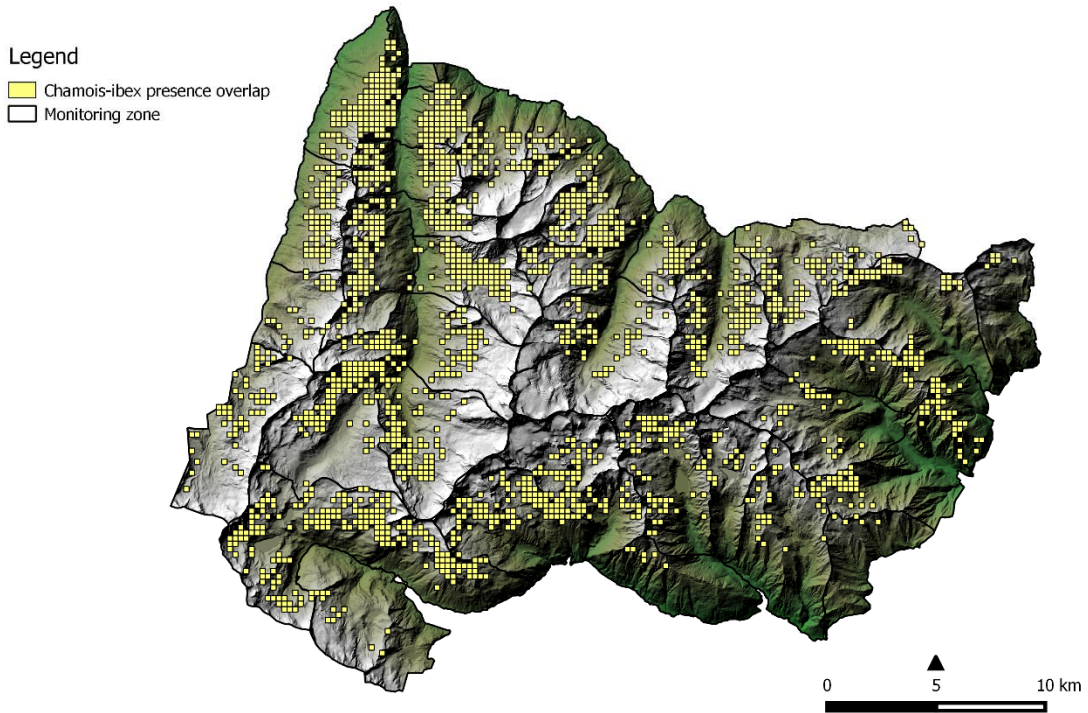CHAMOIS-IBEX OCCURRENCE OVERLAP IN GPNP - SUMMER CENSUS 2000-2013

Fig. 18A. Presence overlap between chamois and ibex considering data census from 2000 to 2013.

3a3 ACTION

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, Italy.

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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).

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 below: the number of successful faecal sample analysed (*N° sampled individuals*), the number of total vegetation food items occurrence (*N° food items*) for both family and species level of analysis.

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	-	-

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 ($\geq$ 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).
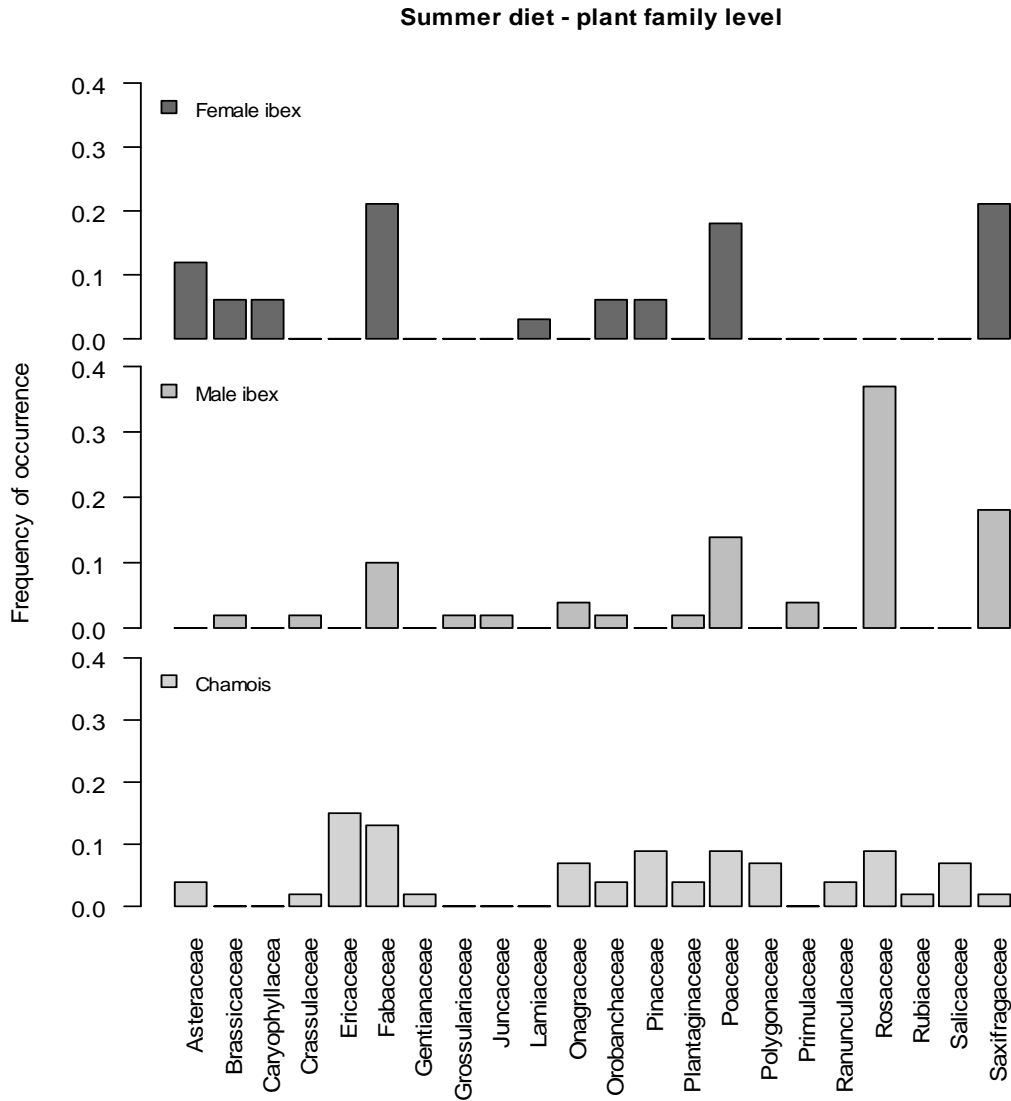
Summer diet - plant family level

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).
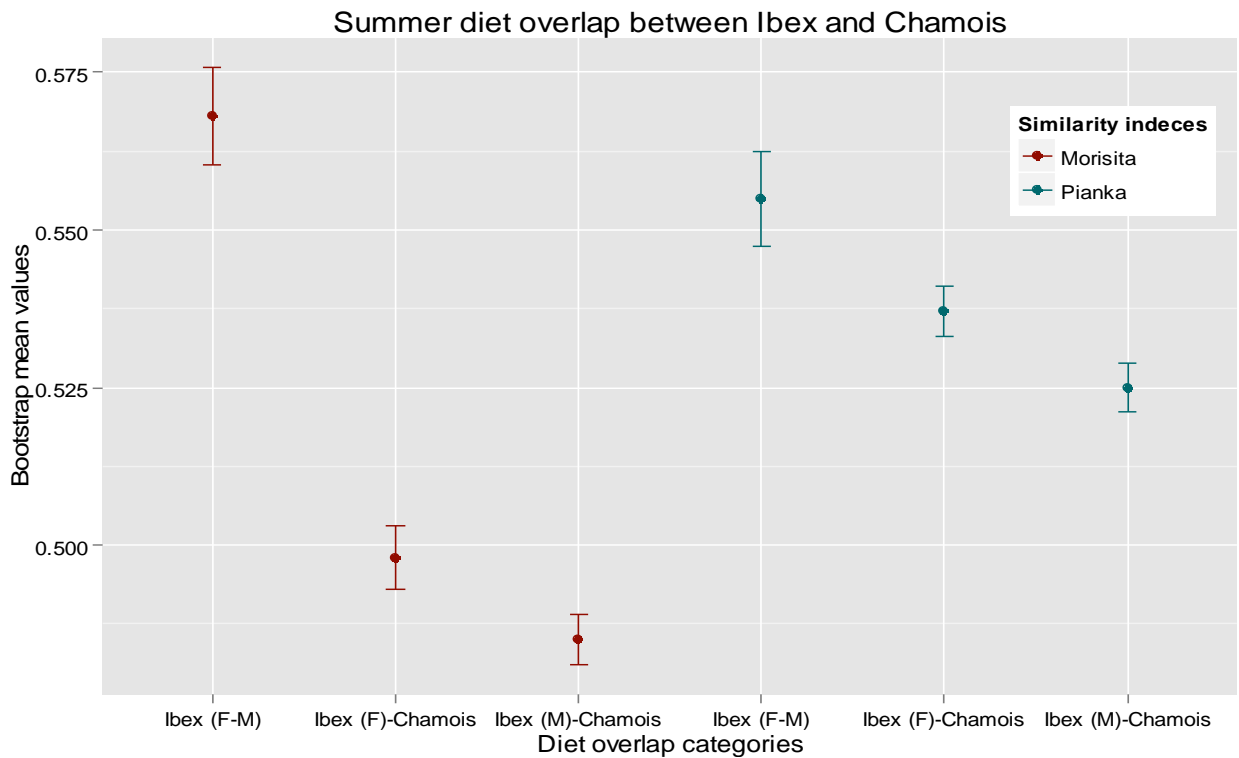
Figure 2. Dietary overlap between ibex (male and female) and chamois at plant family level during summer 2013 in Gran Paradiso National Park. Morisita and Pianka indices bootstrapped values (mean ± SE) for three considered categories: (i) ibex (F-M), female vs. male ibex, (ii) ibex (F) - chamois, female 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 (χ^2 = 57.57, P < 0.001). Cyperacae 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. Cypreraceae not found at family level of diet analysis, with species level here is with the highest frequency of occurrence (Figure 3).
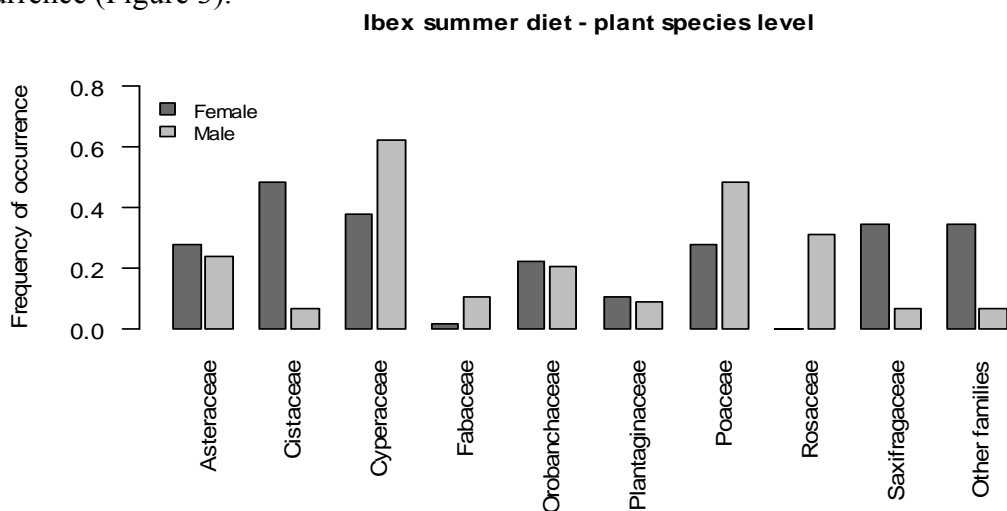

Figure 3. Relative importance of plant families (n=10) based on their frequency of occurrence in the summer diet of female and male ibex, in Gran Paradiso National Park. Families with frequency of occurrence lower than 0.1 were grouped in "Other familes" category.

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 (χ^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).
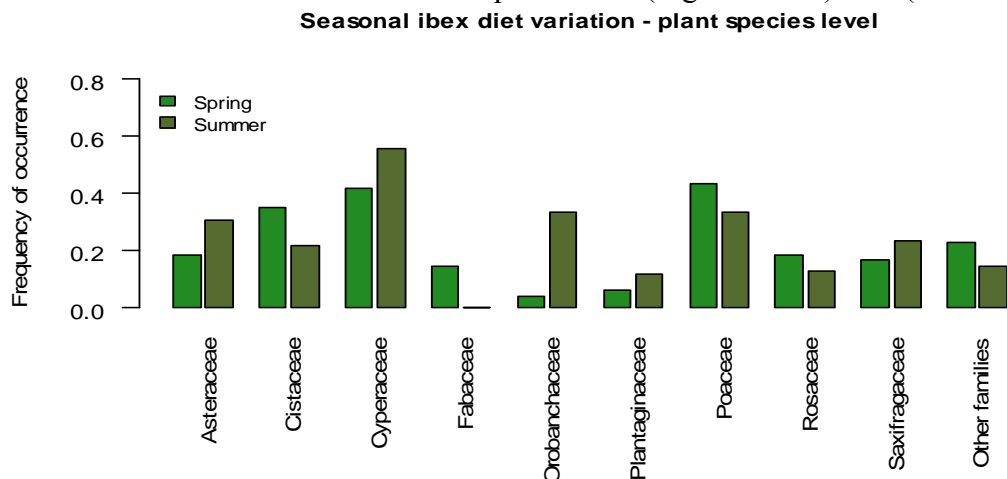

Figure 4. Seasonal ibex diet variation (Frequency of occurrence) at plant species level from Dan barcoding in Gran Paradiso National Park. Families with frequency of occurrence lower than 0.1 were grouped in "Other familes" category. Summer diet was divided in two categories: (i) Spring (May –June) and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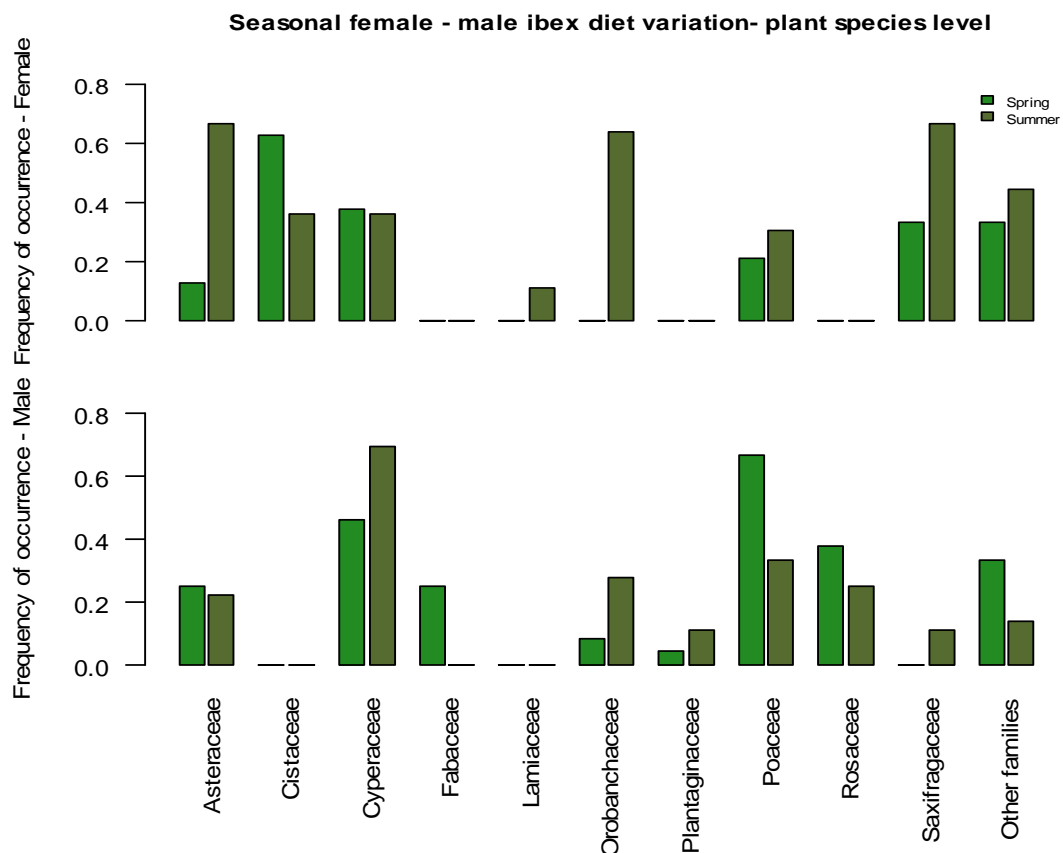

Figure 5. Seasonal ibex diet variation (Frequency of occurrence) at plant species level for female and male ibex from Dna barcoding in Gran Paradiso National Park. Families with frequency of occurrence lower than 0.1 were grouped in "Other familes" category. Summer diet was divided in two categories: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

Discussion

This study confirms the power of Dna barcoding approach to asses ungulate diet compositon (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 quantitavely 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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