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Effects of changing grazing systems on the threatened genus *Peripodisma* (Orthoptera: Acrididae: Melanoplinae) in the Mediterranean mountains of the southern Balkans

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Abstract

This study examined the effects of pastoralism, including cattle grazing, on populations of three species of locally endemic and rare *Peripodisma* grasshoppers in calcareous grassland mountain habitats of northwestern Greece and southern Albania. The three *Peripodisma* species are on the IUCN Red List as near threatened, endangered, and critically endangered species, and cattle grazing had been identified as a key threat to the species. The study sites represented 70% of the known locations of *Peripodisma* genus. The region was historically grazed by local breeds of nomadic sheep and goats, but grazing practices had recently shifted to cattle grazing from non-local cattle breeds. We found a clear relationship between local abundance of *Peripodisma* and overall richness of Orthoptera communities. Orthoptera richness decreased at sites with medium to high impacts of livestock grazing. Cattle grazing had significant adverse effects on overall Orthoptera species richness and on *Peripodisma* abundance. Further studies are urgently needed to gather more data and information to guide grazing management and conservation planning that will provide a more balanced coexistence between livestock and Orthoptera, especially for the rare *Peripodisma* species that are in dire need of conservation management.

Introduction

Pastoral farming in the Mediterranean mountains of Greece and Albania has existed since antiquity. Over time, pastoralism has shaped mountain landscapes and vegetation structure (Kizos, 2008) and, as a consequence, the invertebrate biodiversity. Over the last century, nomadic herding has drastically decreased, evolving into movements of livestock across elevation gradients, with the purpose of ensuring forage availability during summer (Blanc, 1963; Hadjigeorgiou, 2011). Grazing is usually conducted at a low intensity, as sheep and goats are secured at night and shepherded on mountain pastures during the day. Additional changes in pastoralism have emerged in recent decades, particularly in Greece, due to links with the European Economic Community. Non-local cattle breeds have been introduced, resulting in only 0.64% of native cattle breeds (Legakis & Kapari, 2013), due to the new requirement for production of cattle meat (Hadjigeorgiou, 2011). Over this period, there has been a strong decrease in the number of farm holdings, with only a slight decline of overall livestock numbers, resulting in concentrations of herds (Eurostats, 2012). The effects of changes from both overgrazing and undergrazing on vegetation and biodiversity have been object of investigation (Gkoltsiou, 2011; Hadjigeorgiou, 2011). The relationships between vegetation and Orthoptera diversity have previously been demonstrated (Anderson 1964, Kati 2012), and the positive or negative impacts of grazing on Orthoptera species and populations have been studied across a variety of ecosystems and grazing systems (Smith, 1940; Morris, 1969; Holmes *et al.*, 1979; Capinera & Sechrist, 1982; Shiyomi *et al.*, 1982; Grayson & Hassall, 1985; Voisin, 1986; Gueguen-Genest & Gueguen, 1987; Louveaux *et al.*, 1996; Kruess & Tscharntke, 2002; Gonseth, 2010).

Our study investigates the effects of grazing on Orthoptera. Compared to other animal groups, sampling of Orthoptera is relatively simple and identification of most species is easy. Orthoptera are distributed among various trophic levels of the food webs (carnivores, omnivores, herbivores and scavengers), and such trophic relationships are highly dependent upon environmental conditions (*e.g.*, elevation, microclimate, vegetation structure). Orthoptera provide excellent indicators of ecological conditions (Guegen, 1989, 1990, 1996; Kati *et al.*, 2004).

We focused on grasshoppers of the genus *Peripodisma*, which are restricted to the Pindos Mountain Range of Greece and Albania, and were reported to be threatened by domestic livestock grazing (Hochkirch *et al.*, 2016). All three species of *Peripodisma* (*P. tymphii, P. llofizii, P. ceraunii*) are included in the IUCN Red List (Hochkirch *et al.*, 2016). The Tymphi mountain grasshopper *Peripodisma tymphii* Willemse, 1972 is an endangered species (Willemse *et al.*, 2016a) due to its fragmented distribution pattern on seven mountain ranges of south-eastern Europe. The distribution of the Tymphi mountain grasshopper covers the northern Pindos massif in Greece (Willemse, 1972, 1984; Willemse & Willemse, 2008), and the adjacent mountains of southern Albania (Lemonnier-Darcemont *et al.*, 2015; Lemonnier-Darcemont & Darcemont, 2016). The Albanian mountain grasshopper *Peripodisma llofizii* Lemonnier-Darcemont & Darcemont, 2015 is listed as Near Threatened (Chobanov *et al.* 2016), and is known of only four locations in the mountains of the southern Albania region of Gjirokaster (Lemonnier-Darcemont & Darcemont, 2015a; Lemonnier-Darcemont *et al.*, 2015). The Cika mountain grasshopper *Peripodisma ceraunii* Lemonnier-Darcemont & Darcemont, 2015 is a Critically Endangered species (Chobanov *et al.*, 2016) with a very restricted distribution, only known from the Cika mountain in Vlore district of Albania (Lemonnier-Darcemont & Darcemont, 2015b; Lemonnier-Darcemont *et al.*, 2015).

All three species are montane specialists that occur above 1100 m elevation, and have similar habitat preferences (Figure 1). *Peripodisma* inhabit grasslands of Mediterranean montane and alti-Mediterranean vegetation zones, with Cika and Albanian mountain grasshoppers known to favour rock or scree slopes (Lemonnier-Darcemont & Darcemont, 2015a, 2015b). They share similar life cycle characteristics, with egg hatching occurring at the beginning of May, and adults appearing in July through mid-October. These species have been reported to be threatened by domestic livestock grazing, particularly cattle grazing, resulting in declining population trends for the Cika and Tymphi mountain grasshoppers (Chobanov *et al.*, 2016; Willemse *et al.*, 2016). The population trend of the Albanian mountain grasshopper is unknown (Chobanov *et al.*, 2016).

Given that domestic livestock grazing management has recently changed across the region, and the general impression of a strong and quick reduction of biodiversity in impacted areas, the aim of this study is

1. To assess whether the local overall score characterising the richness of orthopteran communities provides a reliable indication of the abundance status of *Peripodisma* populations on these southern Balkan mountains.
2. To identify parameters of pastoralism that have negative ecological effects on the endemic genus *Peripodisma*.

Methods

The taxonomic names and classifications used correspond to the updated Orthoptera Species File Online (Cigliano *et al.* 2018).

Sites and sampling

Sampling of orthopteran communities, including *Peripodisma* species, was conducted in Greece and Albania at nine of the thirteen locations where *Peripodisma* species are known to occur (Figure 2), which represent 70 % of the known localities for the genus. Table 1 provides locality and environmental information for each of the nine sampling sites. Our sampling sites included each of the three type localities where the three species were discovered and described from, two additional sites on the border between Greece and Albania, and four additional sites that were chosen randomly among the remaining eight known sites.

Figure 1. Example of *Peripodisma* habitat of subalpine calcareous grassland (Albania, study site 6).

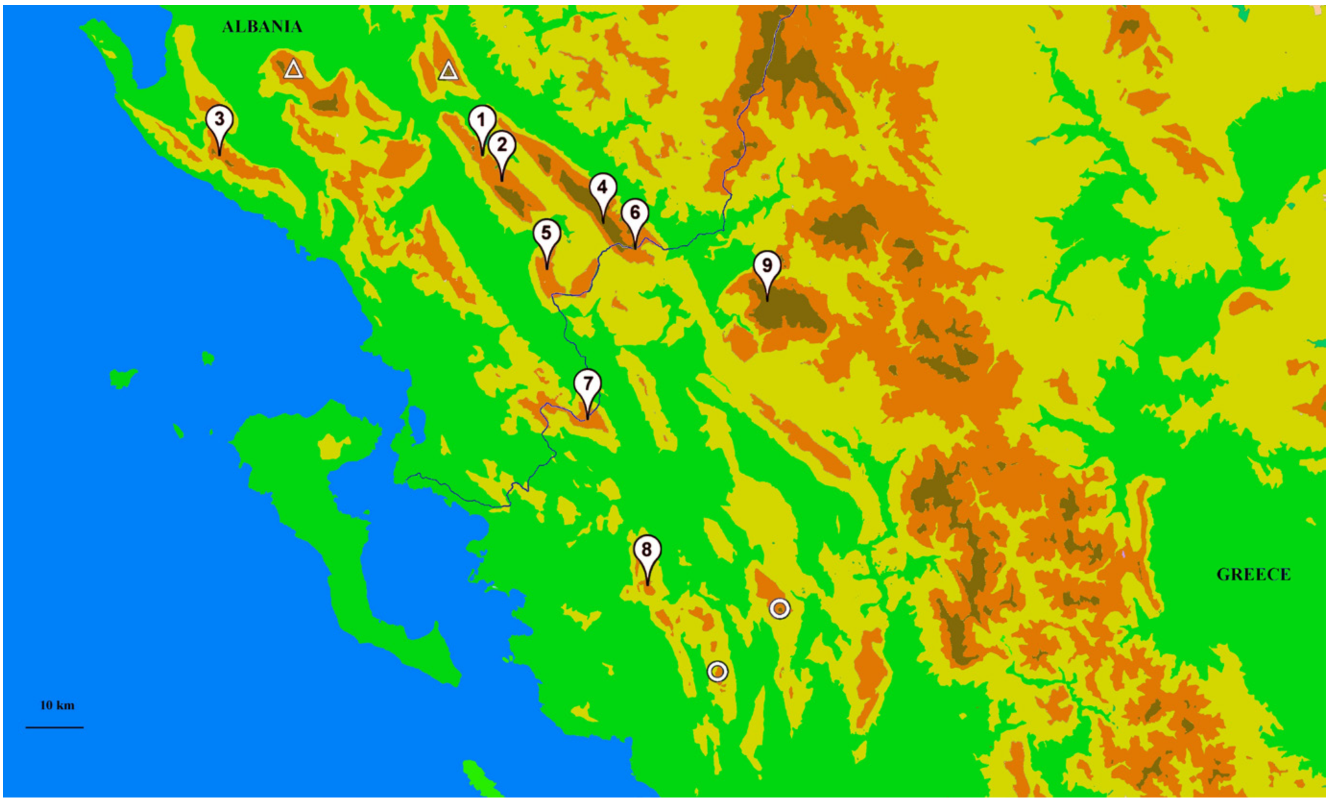

Figure 2. Map of known localities and studied sites. Light green 0-600 m, light brown 600-1200 m, brown 1200-1800 m, dark brown > 1800 m. Markers 1 to 9: studied sites. Triangles: known sites of P. llofizii not studied. Circles: known sites of P. tymphii not studied.

Table 1: Information on sites sampled for this study.

	Locality	Country	Latitude	Longitude	Elevation	Habitat	Peripodisma species
1	Mt Llofiz, Gjirokastër county	Albania	40°12'56.6"	020°09'52.8"	1720 m	Calcareous dry grassland of the Mediterranean mountane stage with *Juniperus sp.* and *Astragalus sp.*	*P. llofizii*
2	Mt Lunxhërisë, Gjirokastër county	Albania	40°10'26.6"	020°12'24.2"	1800 m	Calcareous dry grassland (on rocky ground) of the Mediterranean mountane stage with *Arctostaphylos uva-ursi* and *Astragalus sp.*	*P. llofizii*
3	Mt Qores, Vlorë county	Albania	40°12'48.4"	019°36'17.1"	1830 m	Calcareous dry grassland (on scree) of the Oro-Mediterranean stage with *Juniperus sp.* and *Astragalus sp.*, in edge of open pine forest composed of *Pinus heldreichii*.	*P. ceraunii*
4	W. Mt Nemërçkës	Albania	40°06'19.0"	020°25'19.0"	1750 m	Calcareous dry grassland of the Mediterranean mountane stage with *Arctostaphylos uva-ursi* and *Eryngium sp.*	*P. tymphii*
5	Mt Buretos, Gjirokastër county	Albania	40°01'53.0"	020°18'09.7"	1700 m	Calcareous dry meadow of the Mediterranean mountane stage.	*P. tymphii*
6	Mt Silvit, Gjirokastër county	Albania	40°03'51.2"	020°29'23.0"	1823 m	Subalpine calcareous grassland.	*P. tymphii*
7	Mt Murganës, Gjirokastër county	Albania	39°47'17.8"	020°23'22.7"	1790 m	Calcareous dry meadow of the Mediterranean mountane stage with *Juniperus sp.*, *Astragalus sp.* and *Echinops sp.*	*P. tymphii*
8	Mt Khionistra, Epirus	Greece	39°31'10.0"	020°30'58.3"	1571 m	Calcareous dry grassland with *Juniperus oxycedrus* and *Satureja montana* of the Mediterranean mountane stage.	*P. tymphii*
9	Megalo Papigo, Epirus	Greece	39°58'44.2"	020°46'07.7"	1905 m	Calcareous dry meadow of the Oro-Mediterranean stage.	*P. tymphii*

Pastoralism was present in all known locations were *Peripodisma* species have been found, however there were differences in pastoralism practices between sites.

We sampled Orthoptera during a three-year research period (2014-2016) from the end of July to the beginning of September in each year. This time range matched the main activity period of the adult Orthoptera in the study area. At each sampling site, we studied a large section with homogeneous vegetation structure, exposition, soil, etc. Sampling was always performed during the late morning hours, and only when weather conditions were optimal for diurnal Orthoptera activity: on sunny days with no clouds and reduced wind, and temperature close to the monthly mean temperature (no sampling during heat waves). It was assumed that pastoralism practices did not vary over the three-year study.

Environmental and pastoral parameters

All localities had approximately the same environmental characteristics: calcareous mountain under Mediterranean climate, open grassland landscape above 1500 m elevation. In order to relate variation in Orthoptera species compositions and abundance's to variation in environmental features across the study sites, we focused on parameters which tend to vary considerably across sites. Within each site, the vegetation structure was homogeneous on a very large surface with no detected changes throughout the three years of investigation. At each site, we ascertained vegetation structure and grazing parameters. We recorded the following parameters of vegetation structure: cover (%) of tree layer (> 4 m), shrub layer (1-4 m), dwarf-shrub layer (< 1m), herbaceous vegetation or field layer, as well as bare soil and stones (Prodon, 1988).

Pastoral data were ascertained by direct observation (characteristic of herds (species, breeds) and number of individuals) measured on site directly or by using a drone, and supplemented by personal communications with local shepherds, and using literature data (Caballero *et al.*, 2009; Gkoltsiou, 2011; Hadjigeorgiou, 2011) in order to estimate period and duration of presence of herds on the site, and type of grazing regime. The breeds of sheep, goats and cattle were the same across all sites. We assumed that pastoral conditions were identical from one year to another throughout our study period. We determined a coefficient of relative grazing impact on *Peripodisma* by multiplying the number of individual livestock (all types of livestock; sheep, goats, cattle) per hectare by the number of months of pasture on site, but including different weighting depending on the month of pasture (same weighting for different grazing animals). We considered that the impact of grazing in spring was greater than grazing in summer or autumn because our observations conducted in captivity and in nature showed that the diet of *Peripodisma* consisted mainly of forbs (Figure 3), and the availability of flowers for *Peripodisma* in their early stages appeared to have been a key factor. Preferred forbs were mainly Scrophulariaceae, such as *Verbascum spp*., and also Fabaceae, such as *Astragalus spp*.. When the livestock herds were composed of cattle, they were generally moved to high elevation pastures (habitat of *Peripodisma*) earlier in the season than sheep herds. The cattle consumed the tender plant parts first, such as flowers, which put cattle herds in direct competition for food with *Peripodisma*.

Peripodisma sampling method to measure population density

We used the Linear Indices of Abundance (LIA) method developed by Voisin (1980) to measure the density of *Peripodisma* grasshoppers. Sampling at each site was restricted to an observer walking ten linear 10m transects (1m wide), without overlap, using a 10m-long rope held by the observer. Each transect was randomly sampled (by counting without netting) within the whole pasture area as defined above. The index obtained by the sum of ten transects, resulted in the number of individuals of *Peripodisma* per 100m^2. This sampling was executed first, before any other sampling aimed to measure the overall richness of Orthoptera community, which could have caused movement of *Peripodisma* grasshoppers, and affected subsequent sampling for those species.

Figure 3. *Peripodisma* diet is mainly forbs.

Sampling method to characterize the richness of the Orthoptera community

We walked randomly through each sampling area in all directions, capturing with an aerial insect net all encountered adult Orthoptera until reaching a sample of 100 individuals, then recorded it on a data form to the species rank, and all individuals were then released unharmed. The surface area of any studied site was between five hundred to one thousand square meters. In rare cases where Orthoptera densities were low at a given site, we ceased sampling after two hours regardless of the number of captured individuals (i.e. the total number sampled may not have reached 100).

Sampling without removal has the advantage of greatly limiting disturbance to the orthopteran communities; nevertheless the observer must be quite experienced in order to identify individual orthopteran specimens in the field, and this method has been widely used by others (Dreux, 1962, 1972; Marty, 1968; Defaut, 1978; Luquet, 1985; Voisin 1979, 1980, 1986). This sampling protocol, applicable in open habitats, was appropriate for the sampled sites.

Data collected were used to calculate four ecological indicators of individual abundance and species diversity per locality, which were then used to calculate an overall score characterising the richness of the Orthoptera community at each site, called « overall richness of Orthoptera community » (Lemonnier-Darcemont *et al.*, 2011). The four indices were:

1. Nsc (Number of species capped): Species richness on each site but with a maximum threshold value (maximum 30).
2. Rv (Richness value): Quality of richness of the community. It is the ratio of species with low ecological valence (i.e. narrow ecological amplitude) found on the site to the total number of species recorded, multiplied by 25. This Rv index highlights the local interest of species which are more vulnerable because they are less eclectic in their choices of elective habitats, it presupposes the knowledge of the Species Specialization Index (SSI) as defined

by Julliard et al (2006) of each recorded species. A given species present in h habitat classes among H possible habitat classes has a SSI equal to the square root of (H-h)/h. The SSI reflects the ability of a given species to occupy various habitats, the higher the SSI, the lower the ecological valence of the species. By setting a threshold to the SSI (H/4), we ranked the species in two classes: low ecological valence (high SSI number), and high ecological valence (low SSI number). We used EUNIS biotope codes (Louvel *et al.*, 2013) at first levels to define habitats for SSI computation.

3. En (Equitability indicator): predominance or not of some species. It is the Equitability index (Es) as proposed by Barbault (1992), multiplied by 25. The Equitability index is issued from the index of diversity of Simpson-Weaver (Is = Simpson reciprocal index = 1/D), as Es = (Is-1)/(Ns-1), Ns being species richness. When all species are equally abundant, the indice "En" (= 25 * Es) reaches the maximum value (25), but decreases when the relative abundance of the species is not equally distributed.
4. Rn (Richness of vulnerable species): relative abundance of low ecological valence species. The Rn index is the ratio of number of samples between species with low ecological values and all species. Rn = 20 * vn / (Vn + vn), with vn = Σ ni for species over or equal to the SSI threshold, and Vn = Σ ni for species under the SSI threshold, "ni" being the number of observations of species "i" in the quantitative sampling.

The overall score ranging from 1 to 100, of the richness of the Orthoptera community considered is calculated by summing the four parameters: Nsc + Rv + En + Rn.

Analysis method

For the first objective of the study, the analysis of the correlation between overall richness of Orthoptera community and *Peripodisma* density was performed by a simple Pearson's correlation coefficient computed with the richness per locality and the number of *Peripodisma* sampled per 100 m²; and we provided a plot of the regression analysis by using standard embedded functions of EXCEL. Using XLSAT software, we performed a principal component analysis (PCA) to examine the relationships between environmental parameters, pastoral parameters and Orthoptera species richness, and aimed to provide an ordination of the relationships between key variables.

Results

The richness of the Orthoptera communities (based on relative abundance) and *Peripodisma* abundance (based on quantitative transects) is provided in Table 2. The Pearson correlation analysis between *Peripodisma* densities and the overall richness of Orthoptera community resulted in a significant and high correlation coefficient of $r=0.93$ ($p=0.0004$). A scatter plot from the regression analysis is provided in Figure 4, $R^2=0.856$. These results demonstrate the overall richness of Orthoptera community is correlated with *Peripodisma* densities across the study site pastures, and therefore this score may be used as an indicator of *Peripodisma* density. 86% of variation in *Peripodisma* density is predicted by the score providing the overall richness of Orthoptera community.

Refer to Appendix A for the list of Orthoptera species recorded in the 9 localities visited.

Table 2. Overall score characterising the richness of the Orthoptera community among the study sites, along with population density per 100 m² for *Peripodisma* species.
Nsc = Number of species; Rv = Richness value; En = Equitability among species; Rn = Richness of vulnerable species.

	Orthoptera community					*Peripodisma*
Locality	Nsc	Rv	En	Rn	Richness	Population density
1	13	9.6	15.5	10.4	**49**	**135**
2	8	12.5	18	12.2	**51**	**204**
3	8	6.2	12	2.4	**29**	**26**
4	9	11.1	14.5	10.6	**45**	**127**
5	13	1.9	17.7	2.4	**35**	**85**
6	12	12.5	21.5	10.4	**57**	**230**
7	11	2.3	15	4	**32**	**37**
8	3	8.3	1.2	0.8	**13**	**9**
9	4	12.5	12.2	3.6	**32**	**28**

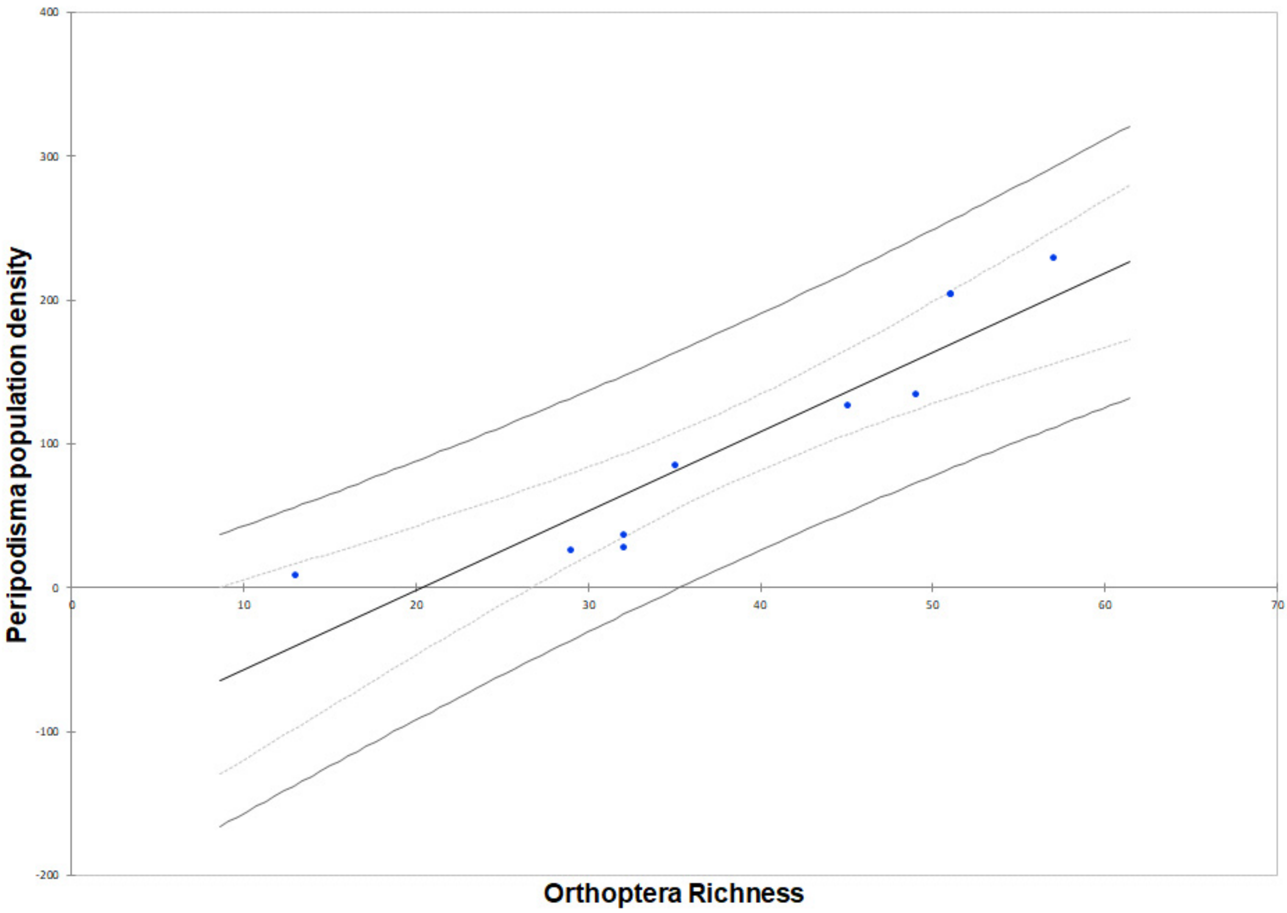

Figure 4. Relationship between measures of abundance of *Peripodisma* and overall index of Orthoptera community richness.

The vegetation structure and pastoralism parameters are provided in Table 3.

The plot of the two main factors of all data recorded is provided in Figure 5. Results of PCA showed a poor correlation between sheep pastoralism and Orthoptera richness and *Peripodisma* abundance, but a clear negative relationship when cattle were present.

Locality 3 is an outlier which can be explained by

Table 3. Vegetation structure and ground cover along with pastoralism parameters across the 9 sites.
NL = non local breed. C = Crossbreed native with non-local breed.

Locality	Vegetation structure and ground cover (% cover)				Pastoralism parameters			
	Shrub layer	Dwarf-shrub layer	Field layer	Bare soil and stones	Herd Composition %		Period	Estimated relative impact
					Sheep	Cattle		
1	_	25	50	25	100		July – mid August	125
2	_	20	30	50	100		July - September	160
3	5	20	20	55	100?	?[1]	June – mid August	525
4	1	20	54	25	100[2]		June - July	460
5	-	15	55	30	100		July - August	355
6	-	15	55	30	100		July – September	80
7	-	15	55	30	50	50 (NL+C)	June – August	550
8	-	30	35	35	20	80 (NL+C)	May - September	1360
9	-	20	30	40		100 (NL+C)[3]	May - September	680

[1] Observed below the study area but no information on their upper elevational limit.
[2] Probably also a small unit of goats in their upper elevational limit (50-100 individuals).
[3] Since 2016 the area occupied by the Peripodisma is no longer grazed by cattle.

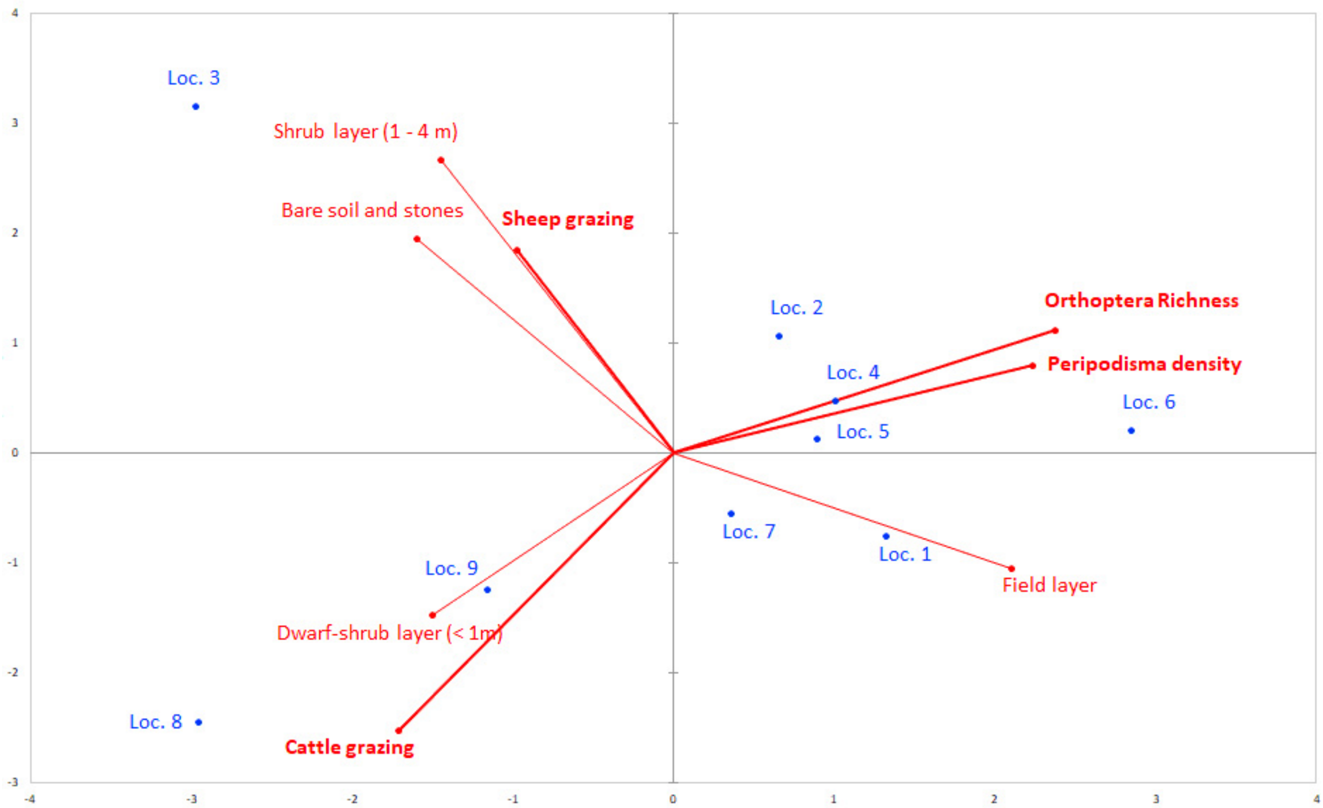

Figure 5. PCA plot on results.

grazing over the past decades. We assume that during past several decades, this locality had been overgrazed. Although the current grazing pressure seems appropriate, the biotope had not yet recovered its potential full biodiversity.

We found a positive relationship between the relative coverage of the herbaceous field layer and the richness and diversity of the Orthoptera communities. However more precise parameters (plant species composition) would probably be necessary to refine correlation parameters.

The results showed that at sites used only by small ruminants (sheep and/or goats), the richness of the Orthoptera communities was high, along with the highest densities of *Peripodisma*, a probable consequence of a current moderate pastoral pressure. In contrast, we measured sharp decreases of *Peripodisma* in areas pastured by cattle of non-indigenous or crossbred breeds (mainly issued from Brown Swiss cows).

Discussion

We found that the densest populations of *Peripodisma* occurred at sites where cattle grazing was absent. Moderate sheep grazing pressure did not seem to be a factor of degradation in those environments, contrary to what we observed when cattle were introduced, at least non-local or crossbreeds (Figure 6), that were not adapted to these Mediterranean mountains. Cattle generally arrived earlier in the season (due to their wider foraging habits) and competed early and directly with phytophagous species of Orthoptera, unlike sheep which were present only in summer on these pastures. This was particularly the case with *Peripodisma*, which prefer to feed on the first buds, young flowers and leaves (unpublished data, Lemonnier-Darcemont), which are also highly desired by cattle.

It is possible that smaller, more rustic local cattle breeds may have less impact on these natural environments (due to smaller body size with less consumption, less soil and vegetation trampling, and a different foraging behaviour). Unfortunately we lack comparative measures to support this assumption because the transition to non-local cattle breeds was completed mainly between 1960 and 1970 (Zervas & Boyazoglou, 1977). After the introduction of breeds of cattle into inappropriate lands (lands very different from the original land of the breed), the process of degradation occurs rapidly through the decrease of the herbaceous field layer. It therefore seems important to be able to measure and monitor grazing impacts as soon as possible, in order to plan for actions that will help to stop this negative trend. Such measures have already been practiced in other ecological regions and environments of northern Greece (Karatassiou *et al.*, 2009).

Figure 6. Crossbreed cattle (Brown Swiss), site number 8

Livestock parasite prevention and other potential treatments for livestock studied for Coleoptera (Lumaret, 2010; Cornille, 2010) could also be considered as a threat to Orthoptera.

Due to its conservation status and its relationship with Orthoptera richness, the use of the genus *Peripodisma* as an indicator genus for the environmental health of grazed montane vegetation formations, seems an appropriate choice. The measure of overall richness of Orthoptera community can be performed more easily and quickly than an accurate measure of the density of *Peripodisma*, therefore it could be used for regular surveys, according to time constraints. Conservation planning and implementation of such plans for these threatened and high heritage value taxa would also provide an effective way to manage the overall biodiversity of montane grasslands of the region.

Our findings presented here should be considered an initial approach for understanding the effects of livestock grazing on Orthoptera species richness and *Peripodisma* species, a snapshot of different localities more or less impacted by pastoralism. We emphasize the importance of planning a comprehensive monitoring protocol targeted at these sites where newly introduced breeds of cattle are apparently adversely impacting *Peripodisma* species. The objective of such research would be to better understand the environmental impacts of cattle and to use that information to plan conservation management strategies for *Peripodisma* and other species that utilize the same montane grassland habitats. The future experimental studies could be performed with different types of grazing treatments, each paired with non-grazed control studies.

Appendix A: Sampled Orthoptera species for each locality.

	1	2	3	4	5	6	7	8	9
Poecilimon gracilioides	X	X				X			
Poecilimon jonicus jonicus					X		X		
Poecilimon zimmeri	X			X		X			
Tettigonia viridissima							X		
Decticus verrucivorus	X			X	X	X	X		
Platycleis affinis affinis	X								
Platycleis grisea			X	X	X				
Platycleis intermedia intermedia							X		
Modestana ebneri ebneri				X					
Parnassiana sp.	X	X		X					
Parnassiana tymphiensis									X
Vichetia oblongicollis					X		X		
Sepiana sepium			X						
Pholidoptera femorata	X			X			X		
Pholidoptera macedonica			X						
Eupholidoptera schmidti			X	X	X		X		
Bucephaloptera bucephala	X								
Psorodonotus macedonicus						X			
Saga hellenica	X	X					X		
Gryllus campestris		X							
Peripodisma ceraunii			X						
Peripodisma llofizii	X	X							
Peripodisma tymphii				X	X	X	X	X	X
Celes variabilis	X	X			X		X	X	X
Oedipoda germanica			X		X				
Arcyptera microptera	X	X							
Stenobothrus fischeri					X				
Stenobothrus lineatus					X				
Stenobothrus nigromaculatus						X			
Stenobothrus rubicundulus	X	X	X		X	X			
Gomphocerus sibiricus						X			
Myrmeleotettix maculatus						X			
Stauroderus scalaris	X			X	X	X	X		X
Chorthippus biguttulus euhedickei					X	X		X	
Chorthippus mollis mollis			X						

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