Published by Associazione Teriologica Italiana
Hystrix, the Italian Journal of Mammalogy
Volume 28 (2): 165–172, 2017
OPEN ACCESS
Available online at:
`http://www.italian-journal-of-mammalogy.it`
doi:10.4404/hystrix-28.2-12332

Research Article

Does ungulate disturbance mediate behavioural and physiological stress responses in Algerian mice (*Mus spretus*)? A wild exclosure experiment

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Keywords:
faecal corticosterone metabolites
physiological reactions
rodents
soil compaction
vegetation cover
ungulate impacts

Article history:
Received: 29 March 2016
Accepted: 16 February 2017

Acknowledgements
We are very grateful to David López Quiroga, Miguel Fernández, Cesar Luis Alonso, Teresa Morán, F. Silveira, L. Martins, A. Muñoz and several students for their help during field work. The authorities and staff of the Cabañeros National Park maintained the ungulate exclosures and allowed us to develop our long-term studies there. In addition, we would like to thank two anonymous referees for their useful comments and suggestions to improve the present work and to Dr. Lucas A. Wauters for his great and generous help reviewing this manuscript. This study is a contribution to the projects ACORNSIZE (CGL2006-06647/BOS), MONTES (CSD2008–00040), RISKDISP (CGL2009-08430) and VULGLO (CGL2010-22180-C03-03). This work was partially supported by the project RiskDisp (CGL2009-08430/BOS) and by a FPU grant from the Spanish Ministry of Education to Á. N-C.

Abstract

Ungulate densities increased recently in Mediterranean oak ecosystem where acorn-dispersing small rodents, like the Algerian mouse (*Mus spretus*), are keystone species. Recent work showed important effects of ungulates on the environment (vegetation and soil characteristics) influencing small mammal behaviour and population dynamics, but little is known on the physiological stress responses of small mammals to ungulates. We studied wild ungulate effects on habitat characteristics and whether ungulate pressure influenced both behavioural and physiological stress responses in wild populations of Algerian mice. We manipulated ungulates' presence by large exclosures in Holm oak *Quercus ilex* open woodland with paired controls, where live trapping of Algerian mice was combined with a detailed evaluation of relevant habitat features for this rodent species such as vegetation height, cover and soil compaction. Further we analysed faecal corticosterone metabolites (FCM) in captured Algerian mice to test whether ungulate presence led to increased glucocorticoids. Fresh faecal samples from 92 different individuals captured with Sherman live traps were collected and analyzed by an enzyme immunoassay. Mouse abundance was higher inside ungulate exclosures due to positive exclosure effects on understory vegetation cover and soil compaction. Mice selected smaller trees with more area covered by resprouts, and this selection was stronger outside than inside exclosures. FCM levels were higher in females than in males. FCM levels were positively correlated with soil compaction and inversely with tree canopy size; however, individuals showed higher FCM levels inside than outside exclosures probably due to the higher local mouse abundance inside exclosures. Mouse behaviour varied in relation to direct effects of wild ungulates on key habitat traits whereas physiological stress responses seemed to be mediated by the increased intraspecific competition, an indirect effect of wild ungulates.

Introduction

Grazing by large herbivores, either wild or domestic, may greatly alter nearly every aspect of the local environment. The ecological effects of grazing include the alteration of the composition of communities, the disruption of ecosystem functions, and the alteration of ecosystem structure (Franklin et al., 1981). Previous studies have shown that ungulates can significantly impact the ecosystem by affecting the floristic species composition, richness, vertical profiles, plant traits, soil erosion and compaction, water infiltration and a number of other attributes (Milchunas et al., 1988; Noy-Meir et al., 1989; McIntyre and Lavorel, 2001; Rodríguez et al., 2003; Torre et al., 2007). Coincident with these vegetation changes caused by ungulates are changes in animal communities. As over-grazing causes important changes in the habitat, ungulates also have the potential to influence the diversity of animal species ranging from insects to birds and mammals (Grant et al., 1982; Bock et al., 1984; van Wieren, 1998; Fuller, 2001; Stewart, 2001; Torre et al., 2007). The effects of ungulates on vegetation and soil mainly affect those animal species that depend on vegetation for food and rely on plant cover and soil for foraging safely and/or for refuge (Milchunas et al., 1988; Keesing, 1998; Eccard et al., 2000). For instance, small mammals are strongly influenced by habitat structure (Rosenzweig and Winakur, 1969; Bowers and Flanagan, 1988) and habitat selection is mainly based on specific components such as favorable microclimates, food resources, avoidance of competitors and cover as protection from predators (Brown et al., 1988; Brown, 1989). Because even moderate grazing by large herbivores reduces vegetation cover drastically, there can be little doubt that small mammal communities could be affected by ungulates (Keesing, 1998; Torre et al., 2007; Muñoz et al., 2009).

Increasing densities of ungulates and the associated increase in grazing pressure may have far-reaching impacts in small mammal ecology. These possible impacts occur through two different ways. Habitat modification or removal due to reductions in cover is assumed to increase the exposure to predators (Birney et al., 1976; Smit et al., 2001; Schmidt and Olsen, 2003; Orrock et al., 2004), and grazing may also influence small mammals directly by physical disturbance such as trampling of soil and browsing (Grant et al., 1982; Bock et al., 1984; Hayward et al., 1997; Torre et al., 2007) and by rooting in the case of wild boar (Focardi et al., 2000); these factors affect food supply, cover and the balance of competition between species. Ungulates may also compete for food with small mammals (Keesing, 1998; Focardi et al., 2000; Caro, 2002; Schmidt et al., 2005) and even predate occasionally on them in the case of wild boars (Herrero et al., 2006). Therefore, clear-cut responses of small mammals to grazing are to be expected.

Most studies on the responses of small mammals to disturbance by ungulates have focused on changes in population (e.g. density) or community (e.g. richness, abundance) parameters in comparisons of captures between grazed and ungrazed areas, showing mixed effects on

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`doi:10.4404/hystrix-28.2-12332`

small mammals according to the species (Grant et al., 1982; Bock et al., 1984; Putman et al., 1989; Heske and Campbell, 1991; Hayward et al., 1997; Jones and Longland, 1999; Jones, 2000; Moser and Witmer, 2000; Giuliano and Homyack, 2004; Valone and Sauter, 2005). However, we have not found available papers on the likely physiological basis of these population or community level responses. Ungulate activity may in fact act as a potential environmental stressor to individual mice, causing physiological stress reactions that could influence population dynamics and behavioral changes (e.g. changing seed predation/dispersal behaviour), and consequently also have an effect on plant population's dynamics.

In mammals, diverse factors such as predators, social conflicts, human disturbances, interspecific competition, and even capture, cause stress reactions (Boonstra et al., 1998; Creel et al., 2002; Sands and Creel, 2004; Barja et al., 2007; Piñeiro et al., 2012; Zwijacz-Kozica et al., 2013). When an animal is subjected to a stressor, glucocorticoid concentrations tend to increase thus indicating a physiological stress reaction (Wingfield and Romero, 2001). Glucocorticoids can be quantified in plasma, urine, saliva and faeces (Sheriff et al., 2011). Faecal glucocorticoid quantification is specially useful as it is a non-invasive method that avoids the additional stress that can be caused by animal capture, handling, sedation and/or transport (Sheriff et al., 2011). In fact, this method has been reported as a useful technique in wild vertebrates such as rodent species (Navarro-Castilla and Barja, 2014; Navarro-Castilla et al., 2014a), North American *Tamiasciurius hudsonicus* and Eurasian red squirrels *Sciurus vulgaris* (Dantzer et al., 2010, 2016), European pine martens *Martes martes* (Barja et al., 2007), wolves *Canis lupus* (Barja et al., 2008), European badgers *Meles meles* (Barja et al., 2012) and wildcats *Felis sylvestris* (Piñeiro et al., 2012). Physiological stress responses to environmental disturbance are adaptive in the short term; however, if glucocorticoid levels remain elevated for longer periods of time ("chronic stress"), a wide range of negative consequences can occur, including immunosuppression, reproductive suppression, and tissue atrophy (Sapolsky, 1992; Sapolsky et al., 2000; Sapolsky, 2002; Stewart, 2003). These negative consequences increase animals' vulnerability, compromising their survival and reproductive capacity (Lochmiller and Deerenberg, 2000) and therefore, affecting their fitness (Möstl and Palme, 2002).

Ungulate densities, either wild or domestic, have increased recently in managed Mediterranean oak ecosystems, the dehesas (Díaz et al., 1997; Perea et al., 2014), where they seem key to explain the general regeneration failure of this high-value system. Negative ungulate effects on regeneration have been proposed to be both direct, by consumption and trampling of acorns and seedlings (Gómez et al., 2003; Pulido and Díaz, 2005; Perea and Gil, 2014a), and indirect, by influencing the effectiveness of acorn dispersal to safe sites for seedlings (Smit et al., 2008, 2009). In fact, the relationship between oaks and mice is a conditional mutualism whose outcome can change to antagonism (i.e. from seed dispersal to see predation) when external factors such as intra- and interspecific competition influence hoarding behavior and cache retrieval (Morán-López et al., 2015, 2016). This latter could be a case of cascading effects of ungulates on oak population dynamics (Schmidt, 2008).

In this study we investigated the impact of wild ungulates on habitat characteristics and on Algerian mice populations in Mediterranean man-made dehesas. In addition, to test whether ungulates had any physiological influence on this rodent species, we collected fresh faecal samples from captured Algerian mice to quantify faecal corticosterone metabolites (FCM) as physiological stress indicators. Given habitat and diet overlap, and given that the shared resources are in limited supply (Tokeshi, 1999), if competition and interaction between ungulates and Algerian mice are important, we hypothesized that: i) Ungrazed ungulate exclosures would provide a better quality habitat with more resources (food and cover) and less compacted soils than available in grazed plots, ii) Rodent abundances would increase in the absence of ungulates and habitat selection by Algerian mice would be affected by habitat traits, consequently, iii) Algerian mice inhabiting the less disturbed ungrazed enclosures would have lower FCM levels.

Materials and methods

Study area

Field work was conducted between November 2010 and February 2012 in the Cabañeros National Park (Ciudad Real province, Central Spain, 39°24′ N, 38°35′ W), established in 1995 as a natural reserve representative of the Mediterranean vegetation of the Iberian Peninsula. Climate is characterized by summer drought (Díaz et al., 2011) with a mean annual rainfall of 636 mm and mean annual temperature is 15.0 °C. Vegetation is dominated by Mediterranean oak forests (*Quercus* spp.) and shrublands. Forests located in the plain lowlands of the park were opened in the 1960s to create dehesas, which are man-made savannah-like woodlands with scattered trees (mean tree density is 14 trees ha^{-1}) growing in an open grassland matrix with almost no shrub cover (<1%; see Pulido et al., 2001; Díaz and Pulido, 2009; Díaz et al., 2011).

Two species of rodents, the Algerian mouse *Mus spretus* and the wood mouse *Apodemus sylvaticus*, inhabit the study area (Muñoz et al., 2009). Both species are prominent predators and dispersers of acorns during the acorn fall season (Pulido and Díaz, 2005; Muñoz and Bonal, 2007). Individual mice positively select oak canopies in dehesas as both refuge areas and foraging grounds, specially during fall-winter, when its staple food are the acorns (Muñoz et al., 2009). Red deer *Cervus elaphus* occupy dehesas and forests at densities of 0.13–0.14 individuals/ha (Jiménez, 2004). Wild boars *Sus scrofa* are also common but at lower densities, 0.8 individuals/ha (Perea and Gil, 2014b), whereas roe deer *Capreolus capreolus* are scarce and restricted to humid pine and oak forests (Díaz et al., 2004). Mammalian carnivores such as common genets *Genetta genetta*, stone martens *Martes foina*, red foxes *Vulpes vulpes*, badgers *Meles meles* and wild cats *Felis sylvestris* are common, as ascertained by scat searches, as were barn owls *Tyto alba*, kestrels *Falco tinnunculus* and harriers *Circus cyaneus*.

Ungulate exclosures

Mice trapping and vegetation and soil measurements were performed in two large ungulate exclosures paired with nearby grazed areas with similar tree density, distribution and cover (see Díaz et al., 2011 for details). Sites were separated by 1500 m. One exclosure was established in 1995 covering an area of ca 150 ha, from which we selected its southernmost 3 ha section including 50 oak trees and an equivalent contiguous grazed area with the same size and shape, also including 50 oak trees. The other exclosure was built in 2008 with 36 oak trees inside 4.65 ha, paired with a grazed area of 4.65 ha with 34 oak trees (see Díaz et al., 2011 for a detailed map). Exclosures were built with wire fences 2 m tall and 32 cm × 16 cm mesh width. Both exclosures allowed free passage by mammalian rodent predators, as ascertained by scat searches inside and outside (Muñoz and Bonal, 2007); avian predators are not excluded by fences.

Animal trapping and data collection in the field

We sampled rodent communities within ungulate exclosures and their paired grazed plots. Four trapping sessions were carried out in November 2010 and 2011 and in February 2011 and 2012, and live trapping was done during new moon to maximize rodent trappability (Díaz, 1992). Traps were located under all trees present in the study sites, as mice barely used the open grassland matrix (Muñoz et al., 2009). A total of 170 oak trees, located inside and outside exclosure plots (details above), were provided with two standard Sherman live traps that were operated during two consecutive nights, with a total effort of 2720 traps-night. Traps were baited with a mixture of tuna and flour and with a piece of apple. Traps were also partly filled with waterproof cotton wool to improve thermal insulation. They were set at dusk and examined the following morning.

Captured individuals were identified to species and weighed (to the nearest 1 g) using a spring balance. Sex and reproductive condition was determined from external characteristics (Gurnell and Flowerdew, 1994). In breeding adult females the nipples on the abdomen and thorax are noticeable and the vaginal membrane appears perforated, whereas breeding adult males present the testicles enlarged quite markedly and usually descended into the scrotal sac. All captured individuals were marked with numbered ear tags to identify and exclude recaptures from data analyses in order to achieve sample independence. Individuals were handled as fast as possible and they were released at the same point of capture. In this research, we fulfilled all the regulations concerning to handling and treatment of animals in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) for animal experiments and manipulations of animals were done under the permit of the authorities of the National park.

Vegetation variables and soil compaction

Measures of vegetation features were done following standardized methods (Newton, 2007; Díaz et al., 2011). The size of the canopy projection of each tree and the area of resprouts under it was estimated by measuring their diameters in N-S and E-W directions. We estimated sizes assuming a roughly circular shape of both the canopy and the resprout patch, as resprouts always encircle tree trunks. Measurements were made only once, in February 2011, since the slow growth of holm oaks (Ogaya and Peñuelas, 2007) would have been within the error margin of the measurement method. Mean height of the resprout patches was measured to the nearest cm with a ruler put vertically on the ground 10 times per patch according to a regular square grid whose size was previously adjusted to each patch size. Local tree density (and its inverse, tree isolation) was measured as the cover of oak canopies in 50-m circles centered on focal trees minus the canopy of such focal trees. Densities were estimated by means of Geographical Information System (GIS) analyses on georeferenced high-resolution (1:5000) aerial photographs. Soil compaction was measured in November 2010 and February 2011 on six regular sampling grids of 10 measurement sites each within each exclosure and in the corresponding adjacent grazed plots. Unfortunately, measurements could not be taken again in November 2011 and February 2012 due to logistic reasons (unavailability of the measurement device; see below). Measurement sites within sampling grids were arranged in two rows of 5 measurement sites spaced 10 m, and grids within exclosures and grazed plots were set every 70 m, thus ensuring that measurement sites were interspersed under tree canopies and in the open grassland to account for microhabitat effects (see Díaz et al., 2011 for details). In each site we measured soil compaction at five points, one in the site and the other four 1 m away in the four cardinal directions. We measured soil compaction at these five points as the force (in N) needed to introduce a steel rod (diameter: 6 mm) with a conical point (length: 6 mm) 20 mm into the soil (Torre et al. 2007). The force was measured with a Mecmesin®BFG 500N electronic force gauge to the nearest 0.1 N. Soil compaction values for the trees occupied by mice were estimated as the mean values of the 25 compaction measures taken in the five nearest measurement sites.

Faeces collection and quantification of Faecal Corticosterone Metabolites (FCM)

Fresh faeces were collected only between sunrise and two hours thereafter to assure that exposure to environmental conditions and microbial action was minimized (Millspaugh and Washburn, 2003; Möstl et al., 2005; Barja et al., 2012) and to avoid the influence of circadian rhythm in excretion patterns (Touma et al., 2003, 2004). Overall, corticosterone peak concentrations in faeces have been observed in related mice species at 9 h after the ACTH injection (range: 8–10 h: Touma et al., 2004; Kalliokoski et al., 2010; Abelson et al., 2016). Therefore, we carried out a judicious trap-monitoring protocol to just collect faeces from individuals trapped less than 8 h. This way, we avoided any possible trapping effect on the physiological stress response, measuring then the stress physiological levels at which Algerian mice actually exist in the field. Faecal samples from traps where urine was detected were excluded to prevent a possible cross contamination. Faecal samples were stored in the freezer at −20 °C until analysis.

FCM were extracted from faecal samples according to the modified method of Touma et al. (2003). Frozen faecal samples were dried at 50 °C until constant weight, and then 0.05 g of dry samples were placed in assay tubes with 0.5 ml of phosphate buffer and 0.5 ml of pure methanol. Tubes were shaken for 16 h and the supernatants were centrifuged at 2500 r.p.m. for 15 min. Pellets were discarded and the faecal extracts were stored at −20 °C until analysis.

For the quantification we used a commercial enzyme immunoassay (ELISA DEV9922; DEMEDITEC) previously validated for measuring FCM in mice by Abelson et al. (2016). The cross-reactivity of the antibodies with other substances according to the manufacturer was less than 2.4%. Previous to the quantification we performed the validation of the present enzyme immunoassay (EIA) kit by carrying out parallelism, accuracy and precision tests (Goymann et al., 1999; Young et al., 2004). A parallelism test of serial dilutions of extracts was performed with dilution ratios of 1:32, 1:16, 1:8, 1:4, 1:2, 1:1, and a curve parallel to those of the standard ($p>0.05$) was obtained. This confirmed that the kit used was reliably and efficiently measuring FCM concentrations in the analyzed faecal samples. Therefore, we did not carry out an ACTH test in order to avoid unnecessary suffering to the animals studied. Recovery (accuracy) was 100.1±34.8% (n=4). Intra- and inter-assay coefficients of variation (precision) were calculated with extracts and the obtained values were 4.7% (n=6) and 8.2% (n=3), respectively. In each assay, we used a standard (50 ng/mL corticosterone) included in the DEMEDITEC kit. When standard corticosterone metabolite concentrations deviated more than 10% from the expected value, the assay was rejected and the samples were re-analysed. The detection limit of the assay for corticosterone was 4.1 ng/mL, and concentrations are expressed as ng/g dry faeces.

Statistical analyses

Exclosure effects on vegetation variables and soil compaction

Site, exclosure and microhabitat effects on soil compaction and on tree characteristics (isolation, tree canopy size, height and cover of resprouts under canopies) were analysed by means of General Linear Models (GLMs) on $\log_{10}$-transformed data (to meet normal distribution criterion). Only significant results from each GLMs have been presented and discussed.

Habitat selection by mice

Resprout height and cover were strongly correlated, so only cover of resprouts was used in the habitat selection analyses since for rodent species vegetation cover seems to be an important factor for habitat selection (Ellis et al., 1997) acting as protection from predators (Orrock et al., 2004). Tree characteristics associated to the presence/absence (dichotomous dependent variable) of mice during trapping sessions were analysed by means of binomial logistic regression models, as most trees were occupied by only one individual. Categorical independent variables were year (winter 2010–2011 or winter 2011–2012), season (November or February), site, and exclosure (inside or outside), and continuous predictors tree canopy size, area covered by resprouts and tree isolation. We tested effects of categorical and continuous predictors and its two-way interactions on tree occupancy by mice as response variable by means of a forward stepwise procedure based on the Wald's statistic with p-to-enter and p-to-remove = 0.05 (Hosmer and Lemeshow, 2000).

Faecal Corticosterone Metabolites (FCM)

We used GLMs (normal distribution) to analyze factors explaining variation in FCM levels (response variable log transformed). Explanatory variables were site, trapping session, exclosure (inside/outside), sex and breeding condition of individuals as fixed factors, whereas body mass, local mouse abundance, tree canopy and soil compaction were included as continuous covariates. We did not include individual trees as random repeated-measures factors in the analyses because a) we did not collect faecal samples from recaptured individuals within the same trapping session; b) no recaptures between trapping sessions were obtained; and c) spatial coincidence of captures of different individuals but in the same trees between different trapping sessions were very scarce (see results below). Because of the significant effect of the exclosures and since the associated local mouse abundance and habitat traits (i.e. tree canopy and soil compaction) differed at both sides (inside/outside), secondary GLMs were conducted separately for inside and outside in order to clarify the influence of each one of these variables studied (i.e. local mouse abundance, tree canopy size and soil compaction). In both cases, FCM levels were the response variable (log transformed), and local mouse abundance, tree canopy and soil compaction were included as covariates. Local mouse abundance was estimated as the number of mice caught in the trees located in 40-m radius circles around trapping sites (Díaz et al., 2011). Results are given as mean ± standard error (SE). We used the SPSS 15.0 statistical software (SPSS Inc, Chicago, IL, U.S.A.).

Results

Exclosure effects on vegetation variables and soil compaction

Results from the GLMs revealed that local tree density did not differ inside and outside exclosures ($F_{1,168}=0.01$, $p=0.946$; 20.4±1.5 trees/ha), and there were no significant interactions between exclosure and site ($F_{1,168}=1.94$, $p=0.166$). Tree size (canopy cover) was not affected by ungulate exclosure ($F_{1,168}=2.92$, $p=0.089$). Resprout height was three times shorter outside exclosures ($F_{1,168}=12.75$, $p=0.0005$; 8.7±0.2 *vs.* 26.8±0.2 cm; backtransformed). The exclosure tended to support higher cover of resprouts around trees on average (1002.70±83.65 cm^2) than outside (826.80±76.80 cm^2), although differences were not statistically significant ($F_{1,168}=2.92$, $p=0.089$). Resprout height and cover were strongly correlated (r=0.833, $p<0.0001$), so that only cover was retained for further analyses. Tree isolation was significantly correlated with both (r=0.217, $p=0.004$ and r=0.226, $p=0.003$, respectively). Canopy size and resprout height showed a significant negative correlation (r=0.226, $p=0.003$).

Exclosure effects were also highly significant on soil compaction ($F_{1,2382}= 61.65$, $p<0.001$), although microhabitat-dependent ($F_{1,2382}=4.21$, $p=0.040$; exclosure x microhabitat interaction). Compaction was 2.7 times higher outside exclosures on average, and it was higher under trees than in the open grassland (1.4 *vs.* 1.2 times higher; Fig. 1). Exclosure effects on vegetation and soil traits did not differ among sites, as shown by non-significant site x exclosure interactions (details not shown).

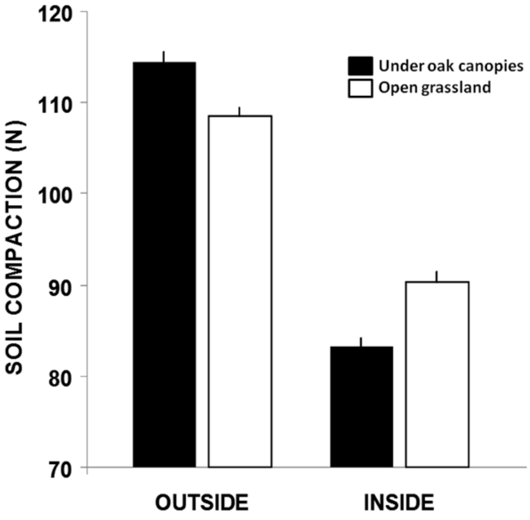

Figure 1 – Mean (+SE) values of soil compaction outside and inside ungulate exclosures according to microhabitats. Filled bars: under oak canopies; open bars: open grassland.

Habitat selection by mice

During the study we captured a total of 177 small mammals (recaptures were excluded), most of them (158; 89%) were Algerian mice (we also caught 18 wood mice and one common shrew *Crocidura russula*, that were not considered further). Abundance of Algerian mice was larger inside exclosures (103 individuals caught inside *vs.* 55 outside), where mice occupied 60% of the trees as compared to 30% outside (Tab. 1). Habitat selection was for smaller trees with more area covered by resprouts, and this selection was stronger outside than inside exclosures (Tab. 1 and Fig. 2). Resprout height did not influence tree occupancy. Significant stronger selection for less isolated trees in the eastern site and for larger trees when more isolated were also found (Tab. 1), but effect sizes for these two later interactions were almost zero as indicated by very low B values. Tree selection did not vary among years or seasonally, and the proportion of trees occupied by mice did not change significantly in time either.

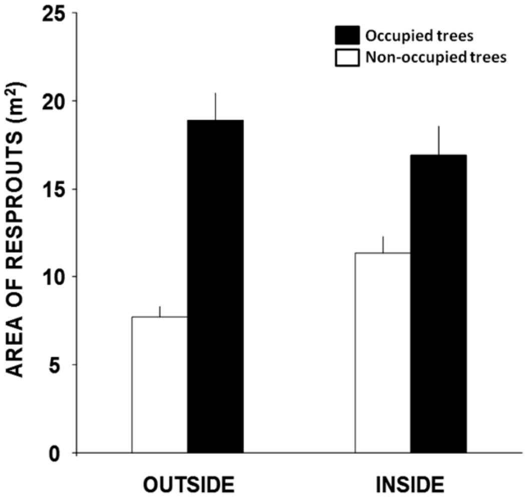

Figure 2 – Mean (+SE) values of area under the tree canopy covered by resprouts for scattered trees occupied (filled bars) and non-occupied (empty bars) by Algerian mice *Mus spretus* either outside or inside ungulate exclosures.

Faecal Corticosterone Metabolites (FCM)

From the 158 Algerian mice captured during live trapping (time of capture <8 h), fresh faecal samples to analyze FCM were available for 92 different individuals (Tab. 2) caught under 80 out of the 170 trees sampled. No individual was recaptured in different trapping sessions, and different individuals were caught under the same trees in different sessions only in 12 occasions (13%). The influence of each factor on FCM levels is represented in Tab. 3. We did not find any evidence of direct effects of the site, trapping sessions, breeding condition or body mass of individuals on FCM levels (Tab. 3). FCM values were influenced by the sex of individuals (females: 33392±4060 ng/g dry faeces, males: 30095±4524 ng/g dry faeces; $F_{1,91}=5.27$, $p=0.026$). Part of the FCM variation was also explained by habitat characteristics. We found effects of canopy size and soil compaction on FCM levels. Individuals living under larger trees (with less height of resprouts under them) and stronger soil compaction showed higher FCM levels (Fig. 3A, B). The exclusion of ungulates also had an effect on FCM levels, individuals captured inside exclosures had higher FCM levels (inside: 33041±3539 ng/g dry faeces, outside: 29225±5488 ng/g dry faeces; $F_{1,91}=9.86$, $p=0.003$). In addition, FCM levels were directly related to the local abundance of mice (Tab. 3). Individuals captured inside exclosures, where abundance of conspecifics was significantly higher, showed higher FCM levels (Fig. 3C). Interactions between factors were not statistically significant, but results indicate potential confounding effects of exclosure as it decreased soil compaction but increased local mouse abundance. In fact, separate analyses of the effects of local mouse abundance, tree size and soil compaction on FCM inside and outside exclosures (Tab. 4) showed that soil compaction influenced FCM levels outside but not inside exclosures, and local mouse abundance affected FCM inside exclosures but not outside them.

Discussion

Overall, Algerian mouse behaviour varied in relation to direct effects of wild ungulates on key habitat traits (i.e. vegetation and soil vari-

Table 1 – Results of the forward stepwise binomial logistic regression (p-to-enter and p-to-remove=0.05) testing whether presence/absence of mice in trees was related to year, season, site, exclosure treatment (categorical predictors), canopy size, area covered by resprouts and tree isolation (continuous predictors) and their two-order interactions.

	Wald statistic	df	*p*	B	SE
Intercept	1.73	1	0.1885	0.46420	0.35302
Site	0.75	1	0.3858	0.40729	0.46959
Isolation	0.50	1	0.4786	-0.00063	0.00090
Area of resprouts	39.42	1	0.0000	0.06597	0.01051
Canopy area	25.37	1	0.0000	-0.04032	0.00801
Exclosure (inside)	16.38	1	0.0001	0.83423	0.20615
Exclosure × resprouts	11.07	1	0.0009	0.03466	0.01042
Site × isolation	12.81	1	0.0003	-0.00087	0.00024
Canopy × isolation	8.25	1	0.0041	0.00002	0.00001

Table 2 – Number of different individuals for which FCM were analyzed in relation to habitat, sex and breeding condition (N=92).

	Sex and breeding condition			
Habitat	Males (N=46)		Females (N=46)	
	Non breeding	Breeding	Non breeding	Breeding
Grazed plots	12	8	9	4
Exclosures	13	13	22	11
Total	25	21	31	15

Table 3 – GLM results testing the effects of different environmental and individual factors on the variation of FCM in Algerian mice (*Mus spretus*).

Factor	df	F	*p*
Site	1	1.485	0.229
Trapping sessions	3	0.826	0.486
Exclosure (Inside/Outside)	1	9.861	0.003
Sex	1	5.272	0.026
Breeding condition	1	2.183	0.147
Body mass of individuals	1	0.166	0.686
Local mouse abundance	1	5.380	0.025
Tree canopy size	1	6.601	0.014
Soil compaction	1	7.018	0.011
Site*Trapping sessions	2	2.536	0.090
Sex*Breeding condition	1	2.935	0.094
Site*Exclosure*Sex	1	3.286	0.077
Error	45		

Table 4 – GLMs analyzing effects of soil compaction, tree canopy size and local mouse abundance on FCM levels inside and outside exclosures.

Exclosures			
Factor	**df**	**F**	***p***
Local mouse abundance	1	4.753	0.034
Tree canopy size	1	2.735	0.104
Soil compaction	1	0.185	0.669
Error	55		
Grazed area			
Factor	**df**	**F**	***p***
Local mouse abundance	1	0.650	0.427
Tree canopy size	1	0.136	0.715
Soil compaction	1	4.643	0.040
Error	29		

ables), whereas physiological stress responses seemed to be indirectly mediated by the effects of wild ungulates on local mouse density and intraspecific competition.

Ungulate exclosure had mainly significant effects on soil compaction and resprout height, but weak effects on the area of resprouts as previously reported (e.g. McIntyre and Lavorel, 2001; Rodríguez et al., 2003; Torre et al., 2007). Ungulate activity greatly increased soil compaction being significantly higher outside exclosures, specially under tree canopies, due to the trampling effect of ungulates that seemed to concentrate their foraging and resting activities under scattered oak canopies as compared to the open grassland (Treydte et al., 2010 and pers. obs.). Since Algerian mice potentially compete with ungulates for food resources, especially acorns, in some periods of the year (Díaz et al., 1993; Focardi et al., 2000; Muñoz and Bonal, 2007), the absence of ungulates inside exclosures could cause an increase of the availability and/or quality of food resources (Smit et al., 2001; Schmidt et al., 2005). Better vegetation characteristics (i.e. greater and higher vegetation cover) together with a lesser compaction of soil which provide availability and easier construction and maintenance of burrows (Khidas and Hansell, 1995; Hayward et al., 1997) could make exclosures a better habitat for small mammals. In our study system, vegetation, soil, and even mice (see below) responses to ungulate exclosure did not differ among two nearby sites excluded 2 and 15 years before samplings. This lack of time-related effects between both sites may have been due to the slow recovery cycles of Mediterranean vegetation (e.g. Doblas-Miranda et al., 2015), that would require longer differences in time from the exclosure to detect them. But also because of the short life cycles of Algerian mice (Palomo et al., 2009), that can react faster to any habitat change.

The abundance of Algerian mice was strongly related to ungulates. Captures increased inside exclosures as a result of ungulates absence, in agreement with previous studies (Torre et al., 2007; Muñoz et al., 2009). Mice occupied twice as many trees inside than outside exclosures. Tree occupation was stable, as indicated by lack of year or seasonal effects. Browsing effects on resprout height were strong, but unlikely to affect small mammals since resprout height did not influence tree selection by mice. It seems that mean height of browsed resprouts (ca. 10 cm) was still large enough to conceal mice against predators (Torre and Díaz, 2004). Selected trees had larger covers of protective resprouts, and this selection for larger covers was stronger outside exclosures. This habitat and tree selection suggests that behavioral responses to habitat characteristics would have reduced direct effects from ungulates (competition for food and/or trampling; Smit et al., 2001; Schmidt et al., 2005; Torre et al., 2007) and indirect effects mediated by predation risk (Jensen et al., 2003; Torre, 2004; Muñoz et al., 2009; Perea et al., 2011).

Based on the outcomes of this study and on previous literature (see references cited above), inter-specific competition for food resources, physical disturbance and facilitation of small mammals' predation seem to be the most important interactions between wild ungulates and Algerian mice. Since environmental changes and disturbing factors can act as physiological stressors for natural populations (Wingfield et al., 1997; Navarro-Castilla et al., 2014a,b) and the impact of ungulates on this rodent species is perceived as acceptable, consideration of possible physiological effects and consequences might be important in order to fully understand the impact of wild ungulates and habitat change on small mammal populations. Our results showed sex of individuals having an important influence over FCM levels, with females showing significantly higher levels than males. This pattern has been reported in other wild rodent species (Touma et al., 2004; Navarro-Castilla et al., 2014a,b) and this difference could be partly due to differences in the metabolism of glucocorticoids between both sexes (Touma et al., 2003). Higher FCM levels in females than in males perhaps could be due to the influence of female sex steroids on the synthesis and release of GCs from the adrenal cortex (Carey et al., 1995). However, in contrast with other studies on rodent species (Dantzer et al., 2010; Navarro-Castilla et al., 2014a,b), we did not find an effect of breeding condition on FCM levels.

The scattered oaks in the grazed plots are important sources of food being frequently visited by ungulates and probably intensifying their impact on rodents (Treydte et al., 2010). Canopy size resulted as an important factor influencing FCM levels. Canopy size was inversely related to resprouts height, so the observed increase in FCM levels could be due to a decrease in protection by resprouts against predators (Preston, 1990; Sheffield et al., 2001; Orrock et al., 2004). Alternatively, although more unlikely, maybe both predators and competitors were attracted to larger tress. Thus, mice could be exposed to a higher risk and competition leading to increased FCM levels. In addition to grazing, other ungulate physical disturbances which also damage burrow systems, such as direct trampling (Hayward et al., 1997; Daniel et al., 2002; Torre et al., 2007) and rooting or predation by wild boar (Focardi et al., 2000; Schley and Roper, 2003; Herrero et al., 2006) could be acting as potential stressors for Algerian mice. Soil compaction cause caverns or changes in water infiltration being directly related to the availability and maintenance of burrows (Khidas and Hansell, 1995; Hayward et al., 1997). Since small rodents, like the Algerian mouse, depend on underground burrows (Khidas and Hansell, 1995), the increase in FCM in more compacted sites detected in trampled grazed plots could be a response of this species to this indirect effect of ungulates on mice (Torre et al., 2007; Muñoz et al., 2009). The lack of local soil compaction effects on FCM inside exclosures, where soils are less trampled and looser, could be interpreted in the same vein.

Despite habitat characteristics and their influence on FCM levels, regarding the effect of exclosures we found somewhat opposite patterns since FCM levels were higher inside. So it seems that potential positive effects of increased food availability and reduced trampling inside exclosures were counter-balanced by other ungulate-mediated effects on individual mice. Thus, local mouse abundance appeared as an important factor positively correlated with FCM levels. Studies on the relationships between population density and adrenal activity have yielded mixed results to date. For some species, increased population densities resulted in an increase in adrenal activity (Goymann and Wingfield, 2004; Raouf et al., 2006), while for other species this relationship was not well supported (Seal et al., 1983; Bradley et al., 1988; Armitage, 1991; Harper and Austad, 2004). In this study, local increases of Algerian mouse abundance were associated with higher levels of FCM inside exclosures, where density was higher, but not outside, where population density was much lower. This finding suggests that social stress may be greater under higher population densities because of increased social contact (e.g. intraspecific competition, increasing aggression rates; Carobrez et al., 2002) and decreased availability of resources (food, refuge and/or partners) which has an important impact on small rodent populations (Lemen and Clausen, 1984; Byrom et al., 2000). Therefore, the high local densities promoted by ungulate exclosure could have led to social stress causing increased FCM levels in the Algerian mouse, as reported for several species (Boonstra and Boag, 1992; Rogovin et al., 2003; Harper and Austad, 2004; Navarro-Castilla et al., 2014a). This increase in FCM levels could be a positive physiological response to cope with increased social interactions associated to high local densities since a recent study on a wild rodent (Dantzer et al., 2013) demonstrated the benefits of increased adrenocortical activity in case of high population density.

To the best of our knowledge this is the first study showing wild ungulates effects on physiological stress responses in small mammals being a significant contribution to the knowledge of environmental factors affecting glucocorticoids levels in wild animals. However, hormone levels in wild ranging animals can be influenced by a wide range of environmental factors and also reflect individual variation (Moberg, 1985, 2000). Therefore, additional research would be needed in order to better understand how mice populations physiologically react and cope with natural competition by wild ungulates and their associated alterations of the surrounding environment.

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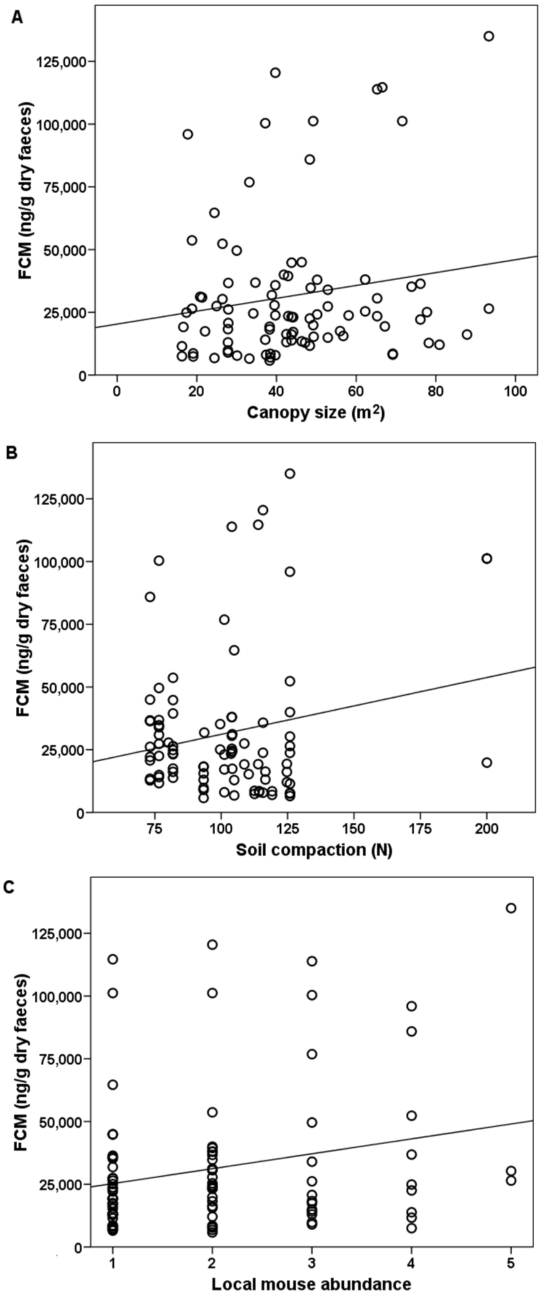

Figure 3 – Relationship between concentrations of faecal corticosterone metabolites (FCM; ng/g dry faeces; mean±SE) and canopy size (A), soil compaction (B) and local abundance of mice (C). Local mouse abundance was estimated as the number of mice caught in the trees located in 40-m radius circles around trapping sites.

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Associate Editor: L.A. Wauters