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No effects of habitat, prey abundance and competitor carnivore abundance on fecal cortisol metabolite levels in wildcats (*Felis silvestris*)

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Conservation physiology is an important tool used to understand how variation in the natural environment can evoke a physiological stress response in free-living animals. The aim of this study was to analyze how fecal cortisol metabolite (FCM) levels vary in response to habitat type, prey abundance and interspecific competition in a free-living population of wildcats in northwest Spain. We collected 110 fresh fecal samples from 25 wildcats along 28 transects between May 2005 and June 2009. To determine habitat characteristics and competing carnivore abundance, we defined 110 circular plots with the fresh wildcat scat at the center. For each plot, we sampled habitat variables, competitor carnivore abundance (pine marten [*Martes martes*] and red fox [*Vulpes vulpes*]) and prey abundance (wood mice [*Apodemus sylvaticus*]). Our results indicate that habitat variables, interference competition and main prey abundance did not significantly affect FCM levels in wildcats.

Introduction

Abiotic and biotic changes are common in the environment, and animals respond to these changes through temporal variation in their vital rates (Morris & Doak 2002) and alterations in their physiological responses (Wingfield *et al*. 1997). Adolph (1956) demonstrated the relationship between physiological regulation and the animal's ability for adapting to new environmental conditions. Physiological tools serve as useful diagnostics in conservation physiology (Wingfield *et al*. 1997, Wikelski & Cooke 2006, Busch & Hayward 2009).

Glucocorticoid (GC) levels are used as an indicator of physiological stress (Wingfield & Romero 2001, Wikeliski & Cooke 2006). When an animal is subjected to a stressor, the hypothalamus releases corticotropin-releasing hormone (CRH), causing the pituitary to secrete adreno-

corticotropic hormone (ACTH), which signals the adrenal cortex to release steroid hormones such as GCs to help overcome stressful situations (Sapolsky *et al*. 2000) and restore homeostasis (Möstl & Palme 2002). However, prolonged exposure to stressors causes chronic increase in GC levels and leads to detrimental 'chronic stress' (Romero 2004). Chronic stress causes depressed immune responses, reduces reproductive success, suppresses growth, or decreases survival, and therefore, it can negatively affect individual fitness (Romero 2004).

Fecal glucocorticoid metabolite quantification is a non-invasive tool that provides important information about endocrine status (Young *et al*. 2004, Palme 2005, Sheriff *et al*. 2011). This method is particularly useful because samples can be obtained without disturbing the animals (Wasser *et al*. 2000) and it has been previously used in wildcat (Piñeiro *et al*. 2012). Thus, analyzing fecal glucocorticoids is a valuable method for studying potential stressors that may affect carnivores under natural conditions (Barja *et al*. 2007, Piñeiro *et al*. 2012).

Habitat conditions may affect GC levels. It is well known that habitat quality can influence the physiology and individual fitness of vertebrates (Huey 1991). In addition, habitat change is considered a stressor in free-living vertebrates (Wingfield *et al*. 1998). Therefore, in the context of landscape ecology, physiological responses can be used to guide conservation and habitat restoration efforts (Wikelski & Cooke 2006). However, few studies have explored the links between physiological responses and habitat spatial patterns (Ellis *et al*. 2012). In mammals, most studies have examined the effect of habitat quality on the levels of GC (e.g., kit fox [*Vulpes macrotis*] and coyote [*Canis latrans*]: Nelson 2005; wolf [*Canis lupus*]: Barja *et al*. 2007; black howler monkey [*Alouatta pigra*]: Martínez-Mota *et al*. 2007; caribou [*Rangifer tarandus caribou*] and moose [*Alces alces*]: Wasser *et al*. 2011).

Food limitation may also elicit physiological stress responses in vertebrates (Ellis *et al*. 2012), affecting survival and limiting growth and fecundity (Boutin 1990). Nutritional stress is defined as a negative physiological and/or behavioural state resulting from the sub-optimal quantity or quality of food available to an animal (Trites & Donnelly 2003). Thus, an increase in GC levels may be attributable to low forage quality (Taillon & Côté 2008) and/or low food abundance (Foley *et al*. 2001).

The effects of interspecific competition may also evoke a physiological stress response (Nelson 2005). There are two types of interspecific competition: exploitation competition and interference competition (Birch 1957). However, there are few studies considering interference competition as a stressor (e.g., habitat segregation: Nelson 2005). The competitive exclusion principle predicts that two species with identical niches cannot coexist indefinitely (Gause 1934). However, subsequent studies revealed that the coexistence of similar carnivore species could be facilitated by different strategies, such as habitat use variation, temporal segregation, or trophic changes (Bonesi *et al*. 2004, Barrientos & Virgós 2006, Hass 2009). In this sense, competition between species would be higher when species whose geographic areas overlap show similar body size and feeding habits (Donadio & Buskirk 2006).

In Atlantic climatic regions, wildcats (*Felis silvestris*) and their competitors, such as the pine marten (*Martes martes*) select forest habitats (Barja 2005b, Klar *et al*. 2008, Pereboom *et al*. 2008). The red fox (*Vulpes vulpes*), which is also a competitor of wildcats, is considered a habitat generalist (Lucherini *et al*. 1995). Wildcat is a territorial species (Sunquist & Sunquist 2002), and its habitat use is often associated with prey availability (Lozano *et al*. 2006). In the north Iberian Peninsula, rodents are the main prey of wildcats and pine martens (Rosellini *et al*. 2008, Piñeiro & Barja 2011), and they are also frequently consumed by the red fox (Carvalho & Gomes 2004). The feeding strategy adopted by wildcat and pine marten is like facultative specialist predators, although the trophic diversity of the pine marten is higher than that of the wildcat (Rosellini *et al*. 2007, Piñeiro & Barja 2011). On the other hand, the red fox is considered a trophic generalist (Jędrzejewski & Jędrzejewska 1992), so the considerable overlap between wildcats and foxes does not necessarily mean high competitive interaction (Carvalho & Gomes 2004). Considering results of the studies mentioned above, the wildcat and the pine

Fig. 1. Location of the study area, Montes do Invernadeiro Nature Park, on the Iberian Peninsula.

marten compete through interference competition that involves direct negative interactions, whereas the wildcat and the red fox are exploitative competitors and show indirect negative interactions. Therefore, interference competition and low prey abundance may act as stressors in wildcat populations.

Thus, in the present study, we examined the physiological stress response induced by the following stressors in free-living wildcats: habitat type, prey availability and interspecific competition with the pine marten and the red fox. We predicted that wildcats would show higher FCM levels in habitats with lower prey abundance and increased interference competition.

Material and methods

Study area

We conducted the study in Montes do Invernadeiro Nature Park, a protected area of 5722 ha, situated in the northwest Iberian Peninsula. The park is located in a transition zone between the Eurosiberian and Mediterranean biogeographical regions (Castroviejo 1977). This study area is mountainous and comprises three landscape systems: scrubland, deciduous forests, and pine forests. Scrubland vegetation is the principal habitat and is formed mainly by Spanish heath (*Erica australis*), prickled broom (*Pterospartum tridentatum*), and yellow rock rose (*Halimium lasianthum*). Patches of deciduous forests are mainly located in valleys and watercourses, where English oak (*Quercus robur*), white birch (*Betula celtiberica*) and English holly (*Ilex aquifolium*) are commonly found. Patches of Scots pine plantations (*Pinus sylvestris*) scattered throughout the study area occupy the remaining land (Pulgar 2004) (Fig. 1).

Transect survey to collect fresh fecal samples

Wildcats, pine martens, and red foxes use forest roads for traveling and frequently defecate on them as a way of visual-scent marking (Robinson & Delibes 1988, Barja *et al*. 2001, Barja 2005a, Piñeiro & Barja 2012). Thus, we surveyed on foot 300-m-long transects along forest roads in order to collect fresh wildcat fecal samples from which we quantified FCM levels. Furthermore, in these transects, we recorded the number of pine marten and red fox scats to estimate their abundances. To increase the probability of locating scats from different individuals and to minimize pseudoreplication, transects were separated by a distance of 700 m. The transect surveys were conducted seasonally between May 2005 and June 2009 in 25 Universal Transverse Mercator (UTM) cells of 1 km^2 each and a total of 28 transects were surveyed monthly. The length of transects was based on individual genotyping performed in our study area, in which we identified 25 wildcat genotypes (6 males and 19 females), with mean home ranges of 953.7 ha for males and 301.2 ha for females (Piñeiro & Barja 2012). Transects were uniformly distributed throughout the study area, and a total area of 311.2 km^2 was surveyed. Morphological characteristics (size and shape) were used to distinguish the scats of the studied species (wildcat, pine marten and red fox) and other carnivores in the study area (weasels [*Mustela nivalis*], stoats [*Mustela erminea*], and wolves). For each pine marten or red fox scat, we registered the following data: species, date and UTM coordinates.

The transects were surveyed between sunrise and three hours thereafter to collect fresh wildcat fecal samples because microbial action and exposure to environmental conditions (such

as heavy rain or snow) can alter the levels of cortisol metabolites (Millspaugh & Washburn 2003). We considered that a scat was fresh when they had a moist layer of mucus, a strong smell and no signs of dehydration (Liu *et al.* 2006). For each fresh scat, we mixed the total fecal sample since GCs are not always distributed equally throughout the scat (Barja *et al.* 2012). We always sampled 10 g of fresh scat to standardize the sampling procedure. All fecal samples were immediately frozen at –20 °C for subsequent laboratory analysis. We recorded the date and UTM coordinates for each fresh wildcat fecal sample. We could not sex all fecal samples because DNA is highly degraded in feces.

Habitat characteristics and competitor abundance

In order to determine habitat characteristics and the abundance of competing carnivores, we defined 110 circular plots, 1 km in diameter, with the fresh wildcat scat samples at the center (Fig. 2). For each plot we analyzed the following variables: (1) pine marten and red fox abundance (by counting their scats), (2) wood mouse abundance, (3) total area of deciduous forests, pine forests, scrublands, and pastureland (pastures and crops), (4) total area occupied by water (rivers and creeks), and (5) total area occupied by forest roads.

Habitat variables, and water and forest road areas in each plot were quantified using a geographical information system (GIS) database (scale 1:25 000) on topographic maps (from Sistema de Información de Ocupación del Suelo en España). Location data were translated into the GIS (gvSIG 1.9. Conselleria d'Infrastrucutures i Transport, Generalitat Valenciana; available at http://www.gvsig.org/web/).

Prey abundance

Wood mice constitute the principal prey of wildcats in the study area (Piñeiro & Barja 2011). Thus, to determine its abundance, we conducted seasonal live trappings at the same time that the transects were surveyed, in the three most representative habitats of the study area (deciduous forests, pine forests, and scrubland) (Fig. 2A). In each habitat, the traps were placed in three UTM cells separated by a mean distance of 3 km. In each cell, we placed 25 Sherman traps in a grid, where traps were located 10 m apart (total effort, 4725 trap nights). Traps remained open 24 h for three consecutive days and they were checked every 12 h. All the traps were baited with bread soaked in oil and insulated from the heat and cold with raw wool. To allow the identification of each individual for later recapture and thus, avoiding pseudoreplication in the abundance data, the captured animals were marked with a non-toxic, waterproof, colored spray paint at different parts of the body. For each captured animal, the colored painting was placed at a different place. Thus, the capture–recapture technique gave us the minimum number of wood mice in each sampled cell. After handling, wood mice were released at the point of capture.

To estimate wood mouse abundance in each plot, we considered its predominant habitat and the season and year in which each fresh wildcat scat was located. Thus, for each plot, we extrapolated the number of wood mice captured at the nearest trapping cell. In addition, we considered that the trapping cell had the same predominant habitat as the plot and that the captures were performed in the same season and year as the collection of the wildcat fecal sample.

Specific origin and individual genotyping

Based on their shape and size, it is difficult to differentiate between wildcat feces and those of feral cats or hybrids. Therefore, it is necessary to use a multi-evidence approach including DNA methods, as reported Davison *et al.* (2002), but *see* also Lozano *et al.* (2013). In the present study, species identification through molecular analysis was conducted using a sub-sample of feces to determine the species of origin and the reliability of the data obtained. In addition, individual genotyping was performed, according to the method used by Oliveira *et al.* (2009), to determine the minimum number of wildcats from which the scats originated; this information was necessary to determine whether the number of

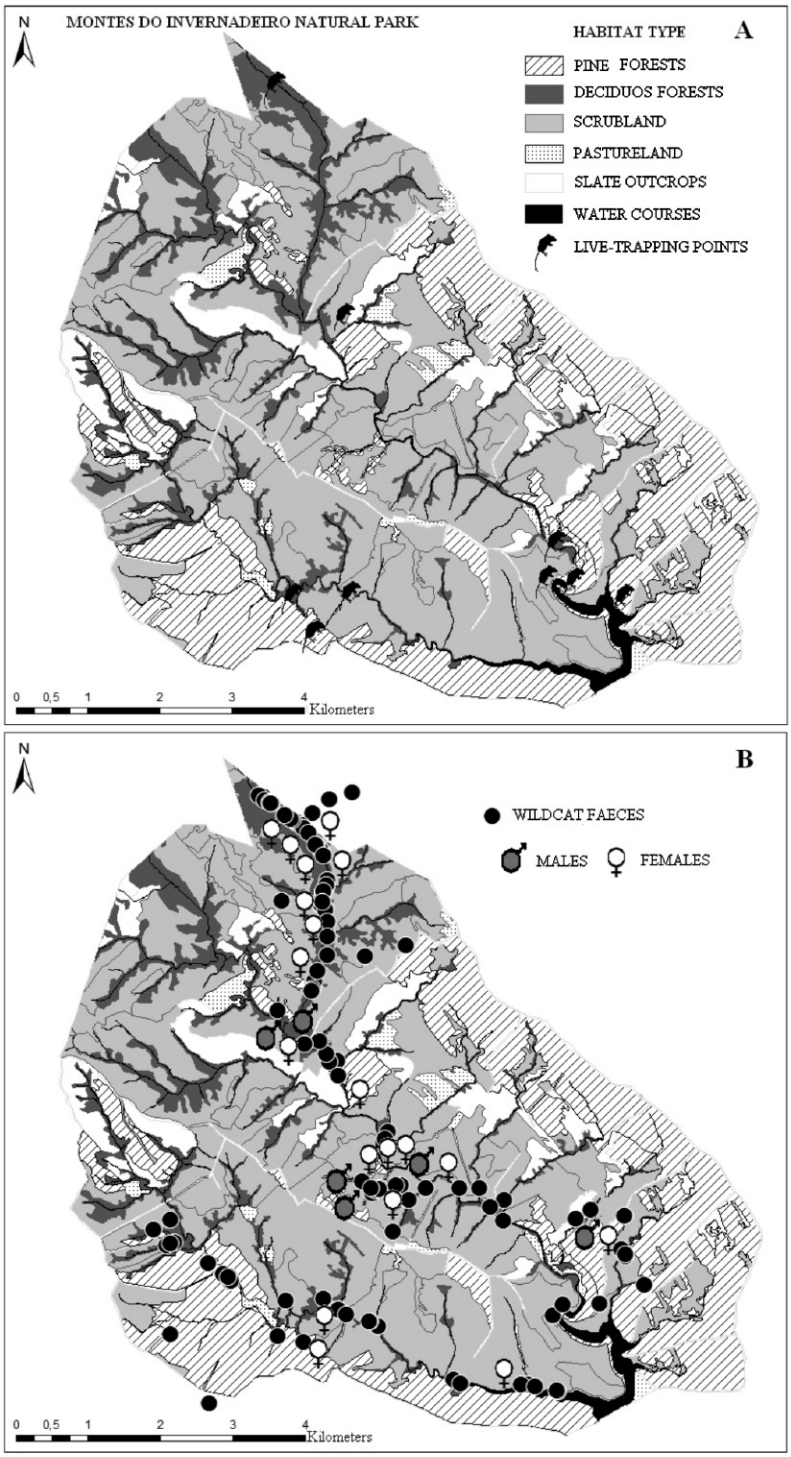

Fig. 2. (**A**) Predominant habitats and trapping points of wood mice in the study area. (**B**) Distribution of fresh wildcat feces collected and the wildcat male and female individuals genetically identified in the study area.

detected scats was representative of the wildcat population. To perform species identification and individual genotyping, we collected a total of 41 fresh fecal samples spread evenly throughout the study area. A total of 25 wildcat genotypes were identified (6 males and 19 females) (Fig. 2B).

DNA extraction from the fecal samples was performed using salting-out and phenol-chloroform extraction (Sambrook *et al*. 1989). We assessed individual multilocus genotypes by using 12 neutral unlinked microsatellites that were isolated and characterized in the domestic cat (Oliveira *et al*. 2008). Polymerase chain reaction (PCR) amplification of individual microsatellites was performed, as described by Randi *et al*. (2001). Allele frequencies, standard diversity indices, and observed (HO) and expected (HE) heterozygosities for each locus and population were calculated using GENETIX 4.05 (Belkhir *et al*. 1996-2004).

To measure the reliability of the scat identifications, we used the Reliotype software (Miller *et al*. 2002), which was used to assess the reliability of the multilocus genotypes and to estimate the number of replicates necessary to obtain a genotype with 95% confidence. The GIMLET software (Valiere 2002) was used to estimate error rates and construct consensus genotypes from the genotyping replicates. Moreover, it was used to regroup identical genotypes from different scat samples and determine parentage between individuals (kinship).

Quantification of fecal cortisol metabolites

Cortisol metabolites were extracted using previously established methods for similar carnivore species (Brown *et al*. 1994, 1996, Young *et al*. 2004, Barja *et al*. 2007). The efficiency of extraction was tested by the addition of a radiolabelled hormone (3H-cortisol, 4000–8000 dpm, ICN, California, USA) to a parallel set of fecal samples. 3H-cortisol evaluates both extraction efficiency and recovery of the technique used. We followed the techniques proposed by others authors (Brown *et al*. 1996, Young *et al*. 2004; and J. Brown unpubl. data). This parameter demonstrates the efficiency of the extraction procedure used, as well as the recovery, since there are several extraction protocols for glucocorticoid metabolites in feces. Schatz and Palme (2001) found differences between sexes in the percentages of radiolabelled cortisol excreted in feces of domestic cats (females: 85%, males: 78.6%). These authors stated that the significance of this observation needs further evaluation. Young *et al*. (2004) performed a validation experiment on domestic cats, cheetah, and clouded leopard (*Neofelis nebulosa*). Then they evaluated results by using a similar cortisol EIA to the one we used our analyses, and concluded that this EIA is suitable for monitoring adrenocortical activity in felids.

The cortisol metabolite concentrations in the fecal extracts were determined using an enzyme immunoassay. Hormone concentrations were calculated using software developed for this technique (ELISA-AID, Eurogenetic, Belgium). Standard dose-response curves were constructed by plotting the binding percentage — $B/B_0 \times 100$ — against the standard hormone concentrations added, where B/B_0 is the relationship between the amount of bound enzyme conjugate to the concentration/mass of the standard/unknowns added.

The mean recovery percentages from fecal extracts were 95% for high concentrations and 98% for low concentrations. Parallel displacement curves were obtained by comparing serial dilutions of pooled fecal extracts with the standard curves. The results showed that both curves were parallel. Intra- and inter-assay coefficients of variation were calculated by assaying 10 replicates of a pooled fecal sample in the same assay and 10 replicates of the same sample in 10 consecutive assays. The percentages recovered for high and low concentrations were 4.5% and 7.5%, respectively. For each sample, cortisol metabolite concentrations were expressed in nanograms per gram (ng g^{-1}) of dry feces. All feces collected were first dried in a conventional oven at 50 °C for several hours, following the procedures described by other authors (Brown *et al*. 1996, Young *et al*. 2004; and J. Brown unpubl. data).

Statistical analyses

Since the quantitative variables were not normally distributed (Shapiro-Wilk test), we $\log(x + 1)$-transformed these variables prior to analyses to ensure normality and homoscedasticity. To avoid multicollinearity (Graham 2003), we performed a principal component analysis

(PCA) on the basis of correlation matrices to reduce the number of habitat variables. Variables included in the PCA were the following: deciduous forest, pine forest, scrubland and pastureland, water, and forest road area.

We used a generalized linear model (GLM) to evaluate the predictor variables (PCA factors, pine marten abundance, red fox abundance, and wood mouse abundance) that influenced FCM levels in wildcats (response variable). In order to select the best model, we ranked the alternative models based on relative differences in the second order Akaike's Information Criterion (AIC). We calculated Akaike's weight (w_i), which is interpreted as the weight of evidence that a model is the best approximating model (Burnham & Anderson 2002). We used the sum of Akaike weights (Σw_i) for each variable to rank variables by their importance (Burnham & Anderson 2002); variables with the highest weight ($\Sigma\omega_m = 0.95$) being more important than the other variables. Selection of candidate models followed the rule in which $\Delta i \leq 2$ has substantial empirical support, where Δi is the AIC difference between model i and the single best model (the model with the smallest AIC value): $\Delta i = AIC_i - AIC_{min}$ (Burnham & Anderson 2002). Also, Burnham *et al.* (2011) following Sugiura (1978) and Hurvich and Tsai (1989) recommend that when sample sizes are small, as they often are in behavioral studies, it is better to use AIC_c instead of AIC. Finally, we checked whether the best models (within 2 AIC_c units from the top-ranked model) improved in terms of AIC with interactions fitted.

Test results whose $p < 0.05$ were considered statistically significant. All analyses were performed with STATISTICA ver. 8.0 software for Windows (StatSoft Inc, Tulsa, USA).

Results

Pine marten and red fox scats were detected in 86 of 110 circular plots containing wildcat scats; the scat number per circular plot ranged from 1 to 7 for both species. We captured a total of 232 wood mice during the study, and their abundance varied between 1 and 25 individuals per circular plot (mean ± SD = 8.9 ± 5.8).

The habitat variable PCA produced two orthogonal factors, which explained 55.1% of the total variance. The first principal component (factor 1) described open areas with high forest road area and low herb cover (water area, forest roads and scrubland areas). The second principal component (factor 2) represented habitats with high herb cover (deciduous forest, pastureland and pine forest area) (Table 1).

The levels of fecal cortisol metabolites were analyzed in 110 fresh fecal samples of wildcats. The GLM showed that the factors considered did not significantly affect the FCM levels (Table 2). A total of 31 occurrence models were possible with the variables considered, but only 12 models were regarded as plausible ($\Delta AIC \leq 2$) (Table 2). Factor 1, pine marten abundance, and factor 2 had the most weight in explaining FCM concentrations (Table 3). By contrast, the abundance of wood mice and red foxes had lower relevance in the occurrence models (Table 3). The relations between wildcat prey abundance or competitor species abundance (pine marten and red fox) and cortisol metabolite concentrations were not significant (Fig. 3). Furthermore, wildcats did not show an increase in FCM concentrations in relation to habitat type, water, and forest road areas.

Discussion

The results of our study indicate that the abundance of carnivore competitors (here the pine marten and red fox), and the abundance of

Table 1. Results of the principal component analysis performed with variables used to describe the wildcat habitat. Asterisks (*) indicate significant correlations ($p < 0.05$) between the variables and factors.

Variables	Factor 1	Factor 2
Water area	0.18	–0.80*
Forest road area	–0.66*	0.21*
Scrubland area	–0.04	0.66*
Deciduous forest area	–0.67*	0.01
Pastureland area	0.59*	0.41*
Pine forest area	0.77*	–0.33*
Eigenvalue	2.07	1.24
Explained variance percenrtge	34.48	20.60

wood mice did not significantly affect the FCM levels in the wildcat. Cortisol metabolite levels in wildcat feces collected in pine forests did not increase significantly. However, an increase in cortisol metabolite levels in wildcats in pine forests was probably because this is one of the preferred habitats of the pine marten (Barja 2005b). These species could compete for the use of space and trophic resources (rodents), which are more scarce in pine forests than in the other habitat types studied here (A. Piñeiro & I. Barja unpubl. data). In addition, snow depth in pine forests is greater (Dötterer & Bernhart 1996), which hinders wildcats when hunting for rodents (Corbett 1979). However, studies with wildcats shows that the species avoid areas with snow (Liberek 1999) and avoids mature forests (Corbett 1979).

The importance of scrubland providing wildcats with cover for shelter and its richness in prey has been shown in several studies (Lozano *et al.* 2003, Thiel 2005, Monterroso *et al.* 2009). In the study area, scrubland is the habitat that harbors a greater abundance of wildcats' main prey, small mammals (A. Piñeiro & I. Barja unpubl. data). Thus, we predicted that in scrubland wildcats would show high FCM levels since competition

Table 2. Relative contribution of each variable in the best models that explain the fecal cortisol metabolite concentrations (response variable) in wildcats. Number of parameters used (k), Akaike information criterion (AIC), difference between each selected model and the best model (ΔAIC), log-likelihood ratio and their respective p values.

Models	k	AIC	ΔAIC	Log-likehood ratio	p
Factor 1	1	186.83	0	0.20	0.65
Pine marten abundance	1	186.90	0.06	0.14	0.71
Factor 2	1	186.92	0.09	0.11	0.73
Red fox abundance	1	186.96	0.12	0.08	0.78
Wood mouse abundance	1	187.04	0.20	0.00	0.95
Pine marten abundance + Factor 2	2	188.05	1.22	0.99	0.61
Factor 1 + Factor 2	2	188.72	1.88	0.32	0.85
Red fox abundance + Factor 1	2	188.76	1.92	0.28	0.87
Pine marten abundance + Factor 1	2	188.77	1.93	0.27	0.87
Pine marten abundance + Red fox abundance	2	188.82	1.98	0.22	0.89
Wood mouse abundance + Factor 1	2	188.82	1.99	0.22	0.90
Red fox abundance + Factor 2	2	188.85	2.01	0.19	0.91

Table 3. Results of the generalized linear models with predictors (Factor 1 and Factor 2: principal component analysis factors, WMA: wood mouse abundance, PMA: pine marten abundance, RFA: red fox abundance) explaining the variation in fecal cortisol metabolite levels. ΔAIC_c represents the difference between AIC_c of the model under consideration (model i) and the one with the lowest AIC_c value of all models, at the top of the table, being $AIC_c = AIC + [2K(K + 1)]/(n - K - 1)$, where K is the total number of estimable parameters in the model. AIC_c weights indicate the strength of evidence that the selected model is the best; w_i ranges from 0 to 1 and provides an effective way to scale and interpret the Δi (Burnham and Anderson 2002). The evidence ratio is calculated as w_0/w_i, where w_0 is the generalized Akaike weight of model 1, and w_i is the generalized Akaike weight of model i.

Model	AIC	ΔAIC_c	AIC_c weights	Evidence ratio
Factor 1	186.83	0	0.14	–
PMA	186.90	0.06	0.13	0.97
Factor 2	186.92	0.09	0.13	0.98
RFA	186.96	0.12	0.13	0.98
WMA	187.04	0.20	0.12	0.96
PMA + Factor 2	188.05	1.37	0.07	0.60
Factor 1 + Factor 2	188.72	2.04	0.05	0.72
RFA + Factor 1	188.76	2.07	0.05	0.98
PMA + Factor 1	188.77	2.08	0.05	0.99
PMA + RFA	188.82	2.14	0.05	0.97
WMA + Factor 1	188.82	2.14	0.05	0.99

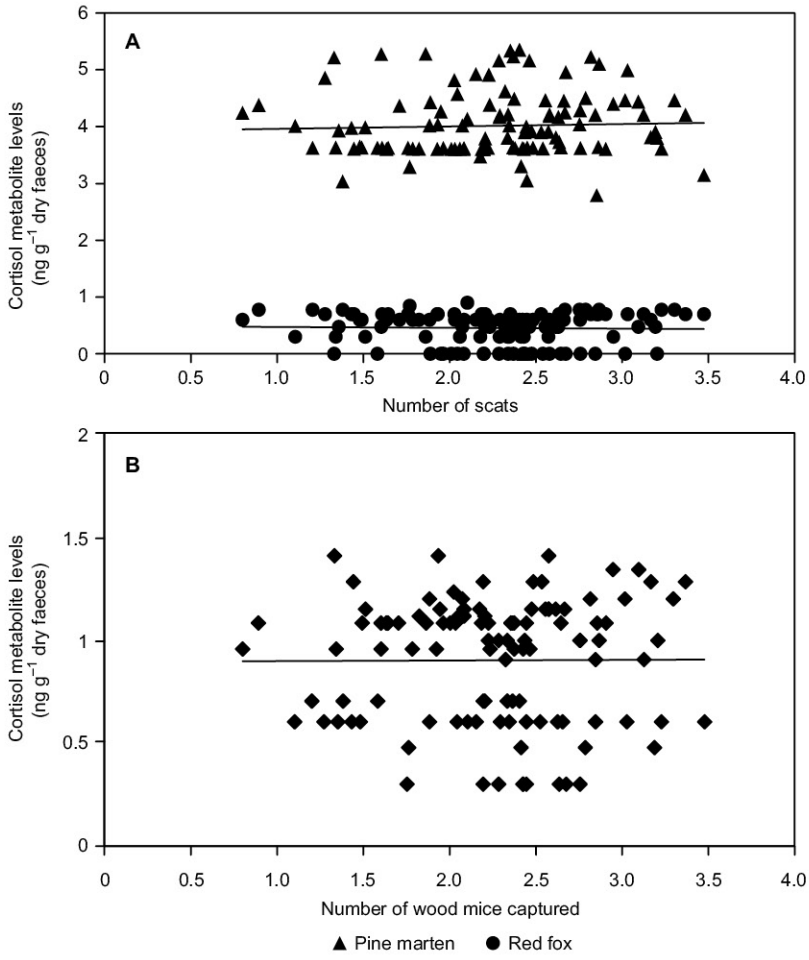

Fig. 3. Comparison of the concentrations of fecal cortisol metabolites [log(x + 1)-transformed] according to (**A**) the pine marten and red fox abundances, and (**B**) wood mice abundance in each plot.

for the main trophic resources could be higher at the intraspecific but also at the interspecific levels because pine martens also seem to hunt frequently in this habitat type (Clevenger 1994). However, we found that scrublands had no statistically significant effect on cortisol metabolite levels in wildcats.

Frequent use of pasture and crop areas by wildcats has been shown in different studies (Easterbee *et al.* 1991, Lozano *et al.* 2003); these areas are mainly used during the night for foraging and hunting (Thiel 2005). Other studies indicated that in these open areas wildcats are exposed to humans (Monterroso *et al.* 2009). However, the results of this study did not concur with those conducted with felids in captivity (Montanha *et al.* 2009) and with that of a study conducted with pine martens in the same study area (Barja *et al.* 2007), in which human presence was considered a stressor. Thus, the increase in FCM levels associated with this habitat may be because these areas are used for hunting but, because wildcats are nocturnal hunters, the effects of the presence of human were too low to show a significant physiological stress response.

The wildcat feces collected from circular plots where deciduous forests and watercourses were the predominant habitats did not show lower cortisol metabolite concentrations than those collected from plots with other predominant habitats. In the study area, deciduous forests are located in valleys with rivers and permanent streams. Deciduous forests provide shelters (Klar *et al.* 2008), e.g., tree cavities, which can be used as dens (Stahl & Artois 1991). In addition, many studies have shown the importance of watercourses in habitat selection for wildcats (Lozano *et al.* 2003, Barja & Bárcena 2005,

Klar *et al*. 2008, Jerosch *et al*. 2010) because of the higher abundance of small mammals (hUallacháin & Madden 2011).

Wildcats use forest roads for travelling and leaving their territorial signals (Corbett 1979, Piñeiro & Barja 2012). Thus, forest roads may act as travel corridors, especially during times of heavy snowfall (Woods & Munro 1996). Also, forest roads could serve as hunting areas, because roads are a suitable habitat for wood mice (Bellamy *et al*. 2000). However, activity patterns of wildcats at forest roads overlap with the activity patterns of the pine marten and red fox (Corbett 1979, Clevenger 1993, Doncaster & Macdonald 1997). Therefore, when there are few forest roads, the probability of encounters with competitors such as pine martens and red foxes is greater and therefore, it was expected that cortisol metabolite levels in feces collected in these zones would be greater too. Nevertheless, forest roads were abundant in our study and also used by tourists visiting the park, so, small mammal abundance in this habitat was expected to be low. Thereby, the use of forest roads as hunting zones does not seem to be sufficiently stressful for wildcats to evoke physiological changes.

In addition to habitat variables, we showed that the abundance of carnivore competitors, such as the pine marten, did not act as an acute stressor in wildcats. The pine marten is a carnivore with slightly smaller body size than that of the wildcat, but with similar prey and habitat preferences (Barja 2005b, Rosellini *et al*. 2008). Therefore, we expected that an increase in FCM levels in wildcats might be related to their defense of resources against a competitor. Donadio and Buskirk (2006) argued that similarity in body size leads competitors to seek similar prey, which increases the likelihood of interference encounters. Furthermore, in our study area, both carnivores (wildcat and pine marten) prey more on wood mice during periods when rodents are easier to capture, but not a too abundant trophic resource (Rosellini *et al*. 2008, Piñeiro & Barja 2011). This could explain why the presence of a competitor, such as the pine marten, does not significantly increase fecal cortisol metabolite levels in wildcats.

In the present study, the abundance of red foxes seemed to have had no influence on cortisol metabolite levels in wildcats. This may be attributable to the generalistic character of the red fox, both in diet (Jedrzejewski & Jędrzejewska 1992) and habitat selection (Lucherini *et al*. 1995); this leads to lower competition between the two species. The abundance of wood mice also did not greatly affect cortisol metabolite levels. These results are consistent with those of a previous study conducted in the same area, where it was found that the consumption of wood mice depended on their ease of capture and not on their availability in the wildcat territory (Piñeiro & Barja 2011).

To the best of our knowledge, this is the first report showing evidence of no effects of habitat type, prey abundance and competitor carnivore abundance on fecal cortisol metabolite levels in wildcats being a significant contribution to the knowledge of environmental factors that can affect physiological stress responses in wild carnivores. Since hormone levels in wild animals can be influenced by a wide range of environmental factors, additional research is needed in order to better understand how animal populations react to ecological factors and natural disturbances.

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