

# Testing the threat-sensitive predator avoidance hypothesis: physiological responses and predator pressure in wild rabbits

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Received: 21 March 2008 / Accepted: 29 September 2008 / Published online: 4 November 2008  
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**Abstract** Predation is a strong selective force with both direct and indirect effects on an animal's fitness. In order to increase the chances of survival, animals have developed different antipredator strategies. However, these strategies have associated costs, so animals should assess their actual risk of predation and shape their antipredator effort accordingly. Under a stressful situation, such as the presence of predators, animals display a physiological stress response that might be proportional to the risk perceived. We tested this hypothesis in wild European rabbits (*Oryctolagus cuniculus*), subjected to different predator pressures, in Doñana National Park (Spain). We measured the concentrations of fecal corticosterone metabolites (FCM) in 20 rabbit populations. By means of track censuses we obtained indexes of mammalian predator presence for each rabbit population. Other factors that could modify the physiological stress response, such as breeding status, food availability and rabbit density, were also considered. Model selection based on information theory showed that predator pressure was the main factor triggering the glucocorticoid release and that the physiological stress response was positively correlated with the

indexes of the presence of mammalian carnivore predators. Other factors, such as food availability and density of rabbits, were considerably less important. We conclude that rabbits are able to assess their actual risk of predation and show a threat-sensitive physiological response.

**Keywords** Fecal corticosterone metabolites · *Oryctolagus cuniculus* · Predator pressure · Threat-sensitive predator avoidance hypothesis

## Introduction

It is crucial for an animal to recognize and respond adaptively to its predators, as predation has strong direct and indirect effects on prey species (Lima and Dill 1990; Kats and Dill 1998; Kraus and Rödel 2004; Apfelbach et al. 2005). Selective pressures have triggered physiological, morphological, and behavioral adaptations in prey species in order to increase the chances of a successful escape (Nilsson et al. 1995; Teplitsky et al. 2005). Generally, the assessment of predation risk is translated into the display of a physiological stress response and an antipredator behavior (von Borell and Ladewig 1992). The perception of a stressful situation activates the sympathetico-adrenomedullary system and the hypothalamic pituitary adrenocortical (HPA) axis (von Holst 1998; Matteri et al. 2001; Möstl and Palme 2002). These increase the levels of catecholamines and glucocorticoids in the blood, which are directed to the mobilization of energy, which in turn is used in the display of a behavioral response (Sapolsky 1992; von Holst 1998). However, antipredator strategies are costly, as they can provoke reduced energy income, energetic investment in defensive structures, or lower mating success (Preisser et al. 2005). According to the threat-sensitive

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Communicated by Jörg Ganzhorn.

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predator avoidance hypothesis, animals should modulate their antipredator responses to the risk of predation perceived (Helfman 1989; Horat and Semlitsch 1994). The threat-sensitive predator avoidance hypothesis has been confirmed in fishes (Kusch et al. 2004; Mirza et al. 2006; Ferrari and Chivers 2006), amphibians (Puttlitz et al. 1999; Teplitsky et al. 2005), and other aquatic species (Loose and Dawidowicz 1994; Kesavaraju et al. 2007), and in most cases in laboratory conditions. As far as we know, the hypothesis has not been tested in mammals.

We tested the threat-sensitive predator avoidance hypothesis in wild populations of European rabbits (*Oryctolagus cuniculus*), subjected to different predator pressures in Doñana National Park (Spain). In Doñana, rabbits constitute the main prey of a wide array of mammalian predators, among them the endangered Iberian lynx (*Lynx pardinus*) (e.g., see Zapata et al. 2007). We analyzed fecal corticosterone metabolites (FCM) of different rabbit populations, which differed in predator pressure. We expected rabbits would show a physiological stress response in accordance to the predator pressure perceived. We used a non-invasive technique, because it is known that trapping and handling have rapid effects on serum corticosterone (Cook et al. 2001) and because in large-scale experiments with wild rabbit populations direct measures are not feasible.

We performed the study at the end of the summer, during the non-breeding season, in order to exclude potential factors that could affect FCM, such as reproductive state, age, or group structure (Goymann et al. 2001; Weingrill et al. 2004; Touma and Palme 2005). During that season agonistic interactions are lower (von Holst et al. 1999), and females are at the same reproductive state. Besides, the structure of the different groups is similar, and there are no juveniles in the groups. Moreover, we analyzed the relative importance of other factors that have been suggested to play an important role in modulating glucocorticoid release (Romero 2002; Wingfield 2005). In particular, we considered differences in food availability and rabbit density among plots. Reduced food availability could result in increased levels of glucocorticoids (Kitaysky et al. 1999). However, we did not expect food to play an important role in our study because in Mediterranean habitats at the end of the summer, food availability is always scarce, and the differences between different areas are small. In social species, such as the European rabbit, high densities of conspecifics may have detrimental effects on vital rates (Rödel et al. 2004a, b), resulting in increased glucocorticoid levels (Nephew and Romero 2003; Goymann and Wingfield 2004; Raouf et al. 2006). We selected a plausible model that could explain the FCM observed by means of an information-theoretic approach. We calculated the relative weight of the variables considered to assess

their effects on FCM. With our study setup, we could account for many of the potential factors that could modify the physiological stress response and test the threat-sensitive predator avoidance hypothesis in wild rabbits.

## Methods

### Study area

The study was carried out in the Doñana Biological Reserve (DBR), the core area of the Doñana National Park, on the right bank of the Guadalquivir River near its mouth in SW Spain (approximately 37°N, 6°30'W). The climate is sub-humid Mediterranean, characterized by dry, hot summers and mild and wet winters. The three main biotopes within Doñana National Park and in the DBR are: sand dunes with pine forest in the dune hollows, scrubland, and marshland. These make up a vegetation mosaic that has been described in several papers (Allier et al. 1974; Rivas-Martínez et al. 1980). The study was carried out in the scrubland biotope, where the predominant vegetation is formed by intermixed hygrophytic scrubland dominated by *Erica* sp. up to 3 m high ([*Erico scoparidae*–*Ullicetum australis* and *Erico ciliaris*–*Ullicetum (minaris) lusitanici* associations] and xerophytic scrubland dominated by *Halimium* sp. up to 1.5 m high (*Halimio halimifolii*–*Stauracanthetum genistoidis* associations).

A rich predator community lives in the area with terrestrial predators such as the Iberian lynx, red fox (*Vulpes vulpes*), Euroasiatic badger (*Meles meles*), Egyptian mongooses (*Herpestes ichneumon*), and European genet (*Genetta genetta*), and aerial predators such as the imperial eagle (*Aquila adalberti*), booted eagle (*Hiaraaetus pennatus*), red and black kites (*Milvus milvus* and *M. migrans*), common buzzard (*Buteo buteo*), tawny owl (*Strix aluco*), and eagle owl (*Bubo bubo*). Some of them are specialists in hunting rabbits (lynx, imperial eagle, booted eagle), but most of them may prey on rabbits when these are abundant (see Zapata et al. 2007).

Since autumn 2004, a recovery plan for rabbits has been carried out in 1,200 ha of scrubland of the DBR, where in 42 different 5-ha plots rabbits were translocated, artificial warrens built, and scrubland managed, if needed, to provide optimal habitat for rabbits (see Román et al. 2006, for details). The shortest distance between any of the plots was 300 m, which is bigger than the average home range diameter of European rabbits in Doñana (20–90 m diameter). Moreover, at the time of the study, rabbit colonies were stable, there was no dispersion, and individuals had small home ranges and core areas (Villafuerte 1994; Lombardi et al. 2007). Therefore, they could be considered independent rabbit colonies. Half of the plots were fenced

(1.80 m high and 0.5 m underground) to prevent or diminish terrestrial carnivores' predation. At the time of this study, rabbits had been translocated into the plots at least 1 year before.

## Data collection

### *Collection of rabbits' fecal samples*

Fecal samples were collected in September 2006 from 20 of these 5-ha plots (10 fenced and 10 unfenced), which a priori should represent different predator pressures and rabbit densities. The samples were collected within a short time interval in the morning (from 08.00 to 09.00). At dawn rabbits start their activity, and fresh fecal samples are easier to detect. Every sample consisted of a rabbit's whole, fresh deposition. From every plot, we collected 25 samples, though in some cases, where rabbit density was lower, the number of samples per plot was lower. On average we collected  $22.9 \pm 3.6$  SD samples per plot. We consider that the number of samples collected, given the density of rabbits in the area, was representative of the local population of rabbits and buffered individual differences (Huber et al. 2003). The pellets were picked up with disposable gloves and collected in sterile eppendorfs. Fresh pellets were identified by their wet and dark appearance, adhesive nature, and their being soft to the touch, which differed from older pellets that usually are dry, lighter, and harder. Thus, we were quite confident that the pellets collected were all from the previous night, and more probably from dawn, when rabbits show an activity peak (Wallage-Drees 1989). Within 1.5 h after collection, all the samples were stored frozen at  $-20^{\circ}\text{C}$  until analysis.

### *Predator pressure*

We focused on mammalian predators because their access to the plots was effectively restricted in the fenced areas, due to the management program, whereas aerial predators freely accessed all the plots. We estimated the actual mammalian predation pressure by sampling the presence of the main carnivores in the study area (Iberian lynx, red foxes, European badger, and Egyptian mongoose; the European genet was excluded since its small size made it very difficult to confidently record their tracks). All those carnivore species might intensively prey on rabbits in the study area (Zapata et al. 2007). Every 2 weeks during the wet season (from November 2005 to May 2006), we looked for tracks on the ground around the five artificial rabbit warrens built in each plot (1.5–2 m from the warren). Doñana has sandy soil allowing easy detection of carnivore tracks during the wet season (Palomares et al. 1998). We did not sample for carnivore tracks during the dry season as

sand is not reliable enough to confidently identify carnivore tracks, mainly those coming from smaller carnivores. Nevertheless, results obtained during the wet season should also be representative of predator pressure during the study since carnivores in the area have a stable spatial and territorial structure [e.g. see Palomares and Delibes (1993) for mongooses, Ferreras et al. (1997) for lynx, or Revilla and Palomares (2002) for badgers]. We calculated an index of terrestrial carnivore predator pressure by summing the averaged number of positive samplings (detection of the species in any of the five sampling points per plot) for each carnivore species. Thus, for the carnivores sampled, the index ranged between 0 (no carnivore detected in any sampling) to 4 (all carnivore species detected in all sampling points). We got an averaged index of 0.10 in the fenced areas, whereas the index of terrestrial carnivore predators was 0.72 in the unfenced areas.

### *Rabbit density*

Rabbit density in the experimental plots was assessed by pellet counts in September–October 2006. This method is a simple and standard method to estimate rabbit abundance in Doñana (Palomares 2001). Pellets were counted in  $0.79 \text{ m}^2$  circle sets ( $n = 25$ ) in a homogeneous  $5 \times 5$  grid inside each 5-ha plot. Four weeks before counting pellets, old pellets were removed from the circles to assure that only the new ones were counted (for further details see Palomares 2001).

### *Food availability*

At the end of the summer, the differences in food availability among the plots used in the study were small, since all were situated in the scrubland habitat and had similar vegetation composition and structure. Nevertheless, plots differ in quality, which is more apparent during the wet season. Therefore, we (1) measured an index of green grass availability for each plot in autumn 2005 (approximately 2 weeks after the first rains in the area) and spring 2006, and (2) checked for consistencies within plots along the time. We visually estimated the green grass cover on the  $0.79 \text{ m}^2$  circles used to count rabbit pellets. The cover of green grass within the circle was estimated by two different observers trained to make similar estimates with the help of known drawn patterns. Due to the fast grass growth, especially in autumn, the samplings were carried out in a time span of only 4 days. With these data we characterized the plots in relation to the food availability as high, medium, or low according to the average and their standard error values obtained from both sampling in each plot. Plots with green grass cover  $< \text{mean} - 2 \text{ SE}$  and those with green grass cover  $\geq \text{mean} + 2 \text{ SE}$  were considered to be

low and high quality ones, respectively. Those in between were labeled as medium quality. There was a significant and positive correlation between green grass availability estimated in both seasons, indicating that plots with higher food availability remained high in all seasons (Spearman:  $r_s = 0.825$ ,  $n = 20$ ,  $P < 0.001$ ).

#### Storage experiment

The time span between fecal deposition and the freezing of the samples may provoke changes in glucocorticoid concentrations (Möstl et al. 1999; Möstl and Palme 2002; Palme 2005). We could not control for deposition time, but we made sure that the feces were fresh, which in a Mediterranean habitat during the dry season means that the feces were recently deposited. However, from the collection of the first sample and the collection of the last one to storing them, about 2 h passed, which could be translated into differences in glucocorticoid levels among the early collected samples and the late ones. We performed a storage experiment in order to control for that time span. For that, ten further samples were collected in a very short time interval (less than half an hour). They were homogenized and divided in five subportions each. One part of every sample was immediately frozen, and every half an hour a further portion was frozen. Therefore, we could control for the 2 1/2 h that could have passed since the first sample was collected until all the samples of the day were frozen.

#### Measurement of FCM

We measured fecal corticosteroid metabolites (FCM) using an already validated method for rabbits (Monclús et al. 2006a). Glucocorticoid metabolites in feces have been proven to be a reliable tool to evaluate adrenocortical activity in many species (Touma and Palme 2005).

The samples were homogenized with mortar and pestle, and 0.20 g of the feces was weighed with a precision balance. The volume was taken to 0.5 ml by adding water. The extraction protocol has been described elsewhere (Monclús et al. 2006a). Briefly, each sample was shaken with 5 ml of methanol (80%) for 30 min on a multi-vortex. Then the samples were centrifuged (2,500g; 10 min), and an aliquot of the diluted supernatant (1:10 with assay buffer) was frozen until analysis. We used an established  $5\alpha$ -pregnane- $3\beta$ ,  $11\beta$ , 21-triol-20-one enzyme immunoassay (EIA), which has been physiologically validated for European rabbits (Monclús et al. 2006a). It is specific for FCM with a  $5\alpha$ - $3\beta$ ,  $11\beta$ -diol structure. For further details of the EIA and its cross-reactions, see Touma et al. (2003; 2004). The interassay and intraassay coefficients of variation were 9.4 and 9.1%, respectively.

#### Water content

For the analysis of FCM we used fresh, wet fecal samples, because the assay was validated with fresh rabbits' feces (Monclús et al. 2006a). In addition, it has not been proven until now whether wet or dry feces are superior for the analysis. Due to the fact that the samples were taken from different areas of the DBR, which may have different water availability, we calculated the water content of the feces. For that we took 0.100 g of seven samples from every plot, dried them in the oven, and calculated the water content. The water content was included in the analysis as a further factor that could affect FCM levels.

#### Data analysis

For analysis, we considered the average FCM values per plot. To determine the factors affecting fecal metabolite concentrations, we used generalized linear models, with a normal distribution and identity link (McCullagh and Nelder 1996). The dependent variable was log transformed. Predator pressure ( $p$ ), water content of the feces ( $w$ ), rabbit density ( $d$ ), and grass availability ( $g$ ) were included as continuous variables. We also included the categorical variable plot ( $f$ ), with two levels, fenced and unfenced, in order to exclude potential fence effects.

For model selection, we followed an information-theoretic approach, considering those models that included at least one of the predictor variables. Akaike's information criterion (AIC) seeks after the model within the candidate set of models that best fits to the data and is the most parsimonious (Burnham and Anderson 2002). In particular, we used the second order Akaike's information criterion (AIC<sub>c</sub>) as Burnham and Anderson (2002) suggested the use of this modification when the relation between the sample size and the number of parameters of the global model is less than 40. We used the modification for overdispersed data, QAIC<sub>c</sub> (Burnham and Anderson 2002). The global model included all the factors. We also considered two two-way interactions: (1) the interaction between food availability and predator pressure, due to the fact that it is considered a synergistic effect (Boonstra et al. 1998; Clinchy et al. 2004), and (2) between food availability and rabbit density, considering that competition will be higher in those patches where food availability is lower. The null model considered that the FCM of rabbits was constant. For model selection we took into account QAIC<sub>c</sub> differences among the different models ( $\Delta\text{QAIC}_c = \text{QAIC}_{ci} - \text{minimum QAIC}_c$ ) and the weights of evidence in favor of each model being the best candidate model ( $w_i$ ). Therefore, the best candidate model should be the one with  $\Delta\text{QAIC}_c = 0$  and the highest weight.

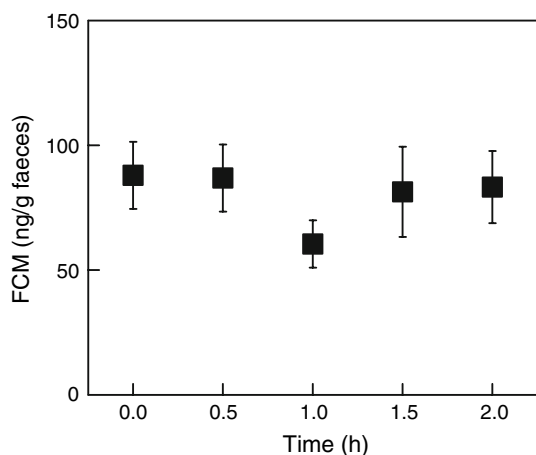
We found a significant positive correlation between the variables water content of the feces and grass availability ( $r = 0.556$ ,  $P = 0.011$ ). We did not consider the models that included both variables together. Similarly, predator pressure and plot were not included together, as they were highly related ( $t_{18} = -9.500$ ;  $P < 0.001$ ). Therefore, our set of candidate models comprised four global models:  $F_{p+w+d}$ ,  $F_{p+g+d+pxg+dxg}$ ,  $F_{f+d+w}$ , and  $F_{f+d+g}$ . For calculating the QAIC<sub>c</sub>, the correction factor used was the one of the model more overdispersed.

We calculated the unconditional parameter estimates ( $\hat{\theta}$ ), the unconditional standard error estimates SE ( $\hat{\theta}$ ), and the relative importance of the variables  $w_{+}(j)$ . For details of the formulas, see Burnham and Anderson 2002. By calculating Nagelkerke’s pseudo  $R^2$ , we assessed the explained variation of the top models of the sets (Nagelkerke 1991). All statistical analyses were performed in STATISTICA 6.0 (StatSoft Inc., USA).

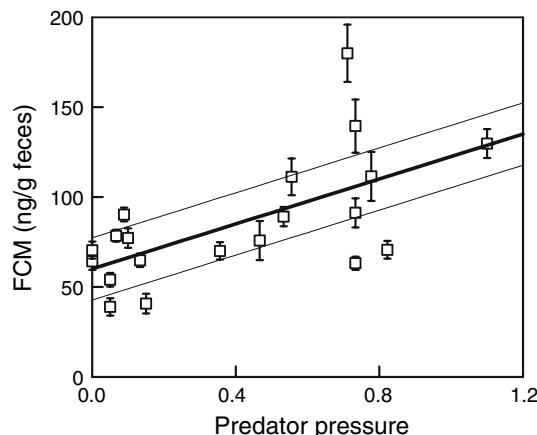
**Results**

With the storage experiment, we did not find overall differences in the FCM among the different time windows (Friedman test:  $\chi^2_{4,10} = 3.798$ ,  $P = 0.434$ ; Fig. 1). Moreover, differences between the concentrations of the first and the last samples, which represented the maximal time span from sample collection to freezing of the experimental samples, were not significant (Wilcoxon matched pairs test:  $Z = 1.120$ ,  $P = 0.263$ ).

Averaged concentrations of fecal corticosterone metabolites (FCM) per plot ranged from 38.9 to 179.9 ng/g feces (mean =  $85.6 \pm 34.5$  SD, CV = 40.3%), and the predator pressure index ranged from 0 to 1.10 (mean =  $0.480 \pm 0.347$  SD) (Fig. 2). Fenced plots reduced the presence



**Fig. 1** Variation of concentrations of fecal corticosterone metabolites (FCM; mean  $\pm$  SE) in relation to the time since the samples were collected ( $t = 0$ ) until they were frozen ( $t = 2$  h after collection)



**Fig. 2** Relationship between FCM values and an index (from track samplings) of predator pressure. The scatter plot represents the observed data (mean  $\pm$  SE). The lines represent the expected values after model selection: the thick line is the parameter estimate and the thin lines the error estimates

of predators. The differences were significant ( $t$  test:  $t_{18} = -9.500$ ;  $P < 0.001$ ). The index of rabbit density was  $0.388$  pellets/m<sup>2</sup>  $\times$  day  $\pm 0.334$  SD (range = 0.010–1.360). Rabbit density was higher in fenced areas ( $t$  test:  $t_{18} = 2.168$ ;  $P = 0.044$ ). The average green grass availability for the two seasons sampled was  $21.67 \pm 3.56$  SD (range = 3.72–60.60) and did not differ between fenced and unfenced plots ( $t$  test:  $t_{18} = -1.044$ ;  $P = 0.310$ ). Finally, the maximum water content of the feces was 66.6%, whereas the minimum was 30.3% (mean =  $41.0\% \pm 8.9$  SD). Fenced and unfenced plots did not differ in the water content ( $t$  test:  $t_{18} = -0.039$ ;  $P = 0.970$ ).

**Analysis of the model**

The goodness of fit considered was  $\hat{c} = 1.43$ . The model with lowest  $\Delta$ QAIC<sub>c</sub> and highest  $w_i$  of the set only considered predation pressure (see Table 1) and explained 41% of the variation (pseudo- $R^2 = 0.410$ ). The model was even better than the one including only the variable “plot” (i.e., fenced vs. unfenced plots). Therefore, it seems likely that potential fence effects are of comparatively lower importance, and as expected, we could show that the main effect of the fence was the enclosure of predators. Considering the other variables, predator pressure was by far the most important variable explaining FCM. Predator pressure was two times better than water content, and four times better than rabbit density (3.9) and grass availability (4.2). Model-averaged estimates and unconditional standard error estimates (Table 2) showed that rabbits subjected to higher predator pressure had higher FCM levels than the ones with low predator pressure (Fig. 2). Moreover, in those samples with a higher water content of the feces, the FCM values were lower.

**Table 1** Set of candidate models (generalized linear models) explaining fecal corticosterone metabolites

Model	$K$	QAIC <sub>c</sub>	$\Delta$ QAIC <sub>c</sub>	$w_i$
$F_p$	4	141.687	0	0.283
$F_f$	4	142.098	0.411	0.231
$F_{f+w}$	5	143.521	1.834	0.113
$F_{p+w}$	5	143.715	2.028	0.099
$F_{p+d}$	5	145.284	3.597	0.045
$F_{p+g}$	5	145.304	3.617	0.045
$F_{f+d}$	5	145.618	3.931	0.038
$F_{f+g}$	5	145.629	3.942	0.038
$F$	3	145.905	4.218	0.033

Factors considered were predation pressure ( $p$ ), plot ( $f$ ), water content ( $w$ ), rabbit density ( $d$ ), and grass availability ( $g$ ). The number of parameters ( $K$ ), the second order quasi-likelihood AIC (QAIC<sub>c</sub> and  $\Delta$ QAIC<sub>c</sub>), and the weights ( $w_i$ ) are given. The models are ordered by  $\Delta$ QAIC<sub>c</sub>. Only the candidate models with  $\Delta$ QAIC<sub>c</sub>  $\leq 4$  and the null model are denoted

**Table 2** Unconditional parameter estimate ( $\hat{\theta}_a$ ); unconditional standard error estimates SE ( $\hat{\theta}$ ) and the relative importance of the variables  $w_{+,j}$  for each parameter affecting fecal corticosterone metabolites

Parameter	$\hat{\theta}_a$	SE ( $\hat{\theta}_a$ )	$w_{+,j}$
Predation	15.809	12.826	0.513
Plot (fenced)	-4.116	3.504	0.444
Water content	-0.075	0.083	0.264
Rabbit density	0.044	0.390	0.133
Grass availability	-0.002	0.007	0.123

## Discussion

Our results confirm that predator pressure was the key factor explaining the physiological stress response of the rabbits during the non-breeding season. Rabbits in plots with high predator pressure had FCM levels up to four times higher than rabbits living in plots with low predator pressure. Moreover, we showed the adjustment of the physiological stress response of rabbits under field conditions provoked by the predator pressure experienced. These results support the threat-sensitive predator avoidance hypothesis in a mammal under field conditions.

Other experiments under field conditions have failed to detect a physiological stress response when animals were exposed to predators (Cockrem and Silverin 2002; Monclús et al. 2006b), whereas under laboratory conditions animals showed an increase of corticosterone levels (Cockrem and Silverin 2002; Monclús et al. 2005, 2006a). These studies simulated the presence of predators and probably the lack

of reinforcement through direct encounters with predators could be responsible for the absence of a physiological stress response. Thus, at least for rabbits, we can conclude that when they only encounter predator odor, they do not display a physiological stress response (Monclús et al. 2006b), whereas the opposite is found when they are in direct contact with predators. In our study, predator pressure was the result of different predators acting in the same plots. Competition among predators would result in different visit times, and therefore, the rabbits in the plots with higher predator pressure index values would suffer higher encounter rates. Aerial predators might have similar consequences on FCM values. The main aerial predators in the area are buzzards and red kites (C. Yuste, personal communication), which are common predators of juvenile rabbits (Veiga and Hiraldo 1990; Mañosa and Cordero 1992). Although buzzards and kites do not normally prey on adult rabbits, which were the only age class present at the moment of the study, it would be interesting to take them into account in further studies.

According to the threat-sensitive predator avoidance hypothesis, prey should adjust their antipredator responses in relation to the risk assessed (Helfman 1989; Ferrari and Chivers 2006), and probably this adjustment is driven by the stress response displayed. The hypothesis has mainly been tested behaviorally, whereas in our study we used physiological stress responses as a measure of the animals' assessment of the risk of predation (Horat and Semlitsch 1994; Puttlitz et al. 1999; Foam et al. 2005; Pollock et al. 2005; Ferrari and Chivers 2006). Under field conditions, the study of the behavior of wild animals is not always feasible, and physiological measures could be an appropriate tool to monitor the assessment of the risk of predation. Indeed, it has been shown that such psychological stressors stimulate the stress response in a linear way (Sapolsky 1992), and physiological responses and behavior are positively correlated (von Borell and Ladewig 1992; Dufty et al. 2002). By means of such a fine activation of the HPA axis, animals can obtain the energy required to develop a suitable anti-predator behavior. For instance, Atlantic salmon (*Salmo salar*) increased ventilation rate according to predation risk and predator density (Hawkins et al. 2007).

Food availability only had a weak effect on FCM in our study in spite of the results shown in other studies (Bonstra et al. 1998; Kitaysky et al. 1999; Clinchy et al. 2004; but see Lanctot et al. 2003). The model that included this variable was six times less likely than the best model and was more parameterized. At the end of the summer in Mediterranean habitats, green grass is always scarce, and probably the predictability of the situation (i.e., at that time of the year high quality food is always scarce) could

explain the small effect of food on this study (Pravosudov et al. 2001; Landys et al. 2006).

The density of rabbits did not play an important role in explaining the observed FCM, even though we encountered important differences among plots. High densities have been highlighted as a major factor provoking increased glucocorticoid levels in several species (Goymann and Wingfield 2004; though see Raouf et al. 2006). In larger groups, higher density contact is usually related to increased aggression rates and social instability (Kotrschal et al. 1998; Deviche et al. 2000; Carobrez et al. 2002; Rogovin et al. 2003; Greives et al. 2007). In rabbits, social instability is mainly associated with the breeding season. The social system of the rabbit is characterized by sex-specific linear rank hierarchies, and intrasexual aggression reaches the maximum at the beginning of the breeding season, when the social ranks are established among the members of the social group (von Holst et al. 1999). During the non-breeding season, when our study was carried out, aggressions are scarce, and direct density-dependent factors should play a minor role, which might explain the relatively small impact of rabbit density on FCM levels.

Finally, an important methodological issue arising from our study is related to the water content of the feces. The model that included the variable water content together with predator pressure was the second best of our candidate models. Those samples with higher water content were the ones with lower FCM. Although water content should not be an important confounding variable in experiments, where housing and feeding conditions of animals are standardized (e.g., laboratory or zoo animals, domestic livestock), it should, however, be taken into account, when the fecal samples come from field studies.

In general, our results support the threat-sensitive predator-avoidance hypothesis: European rabbits under field conditions were able to detect slight changes in predator pressure, and these are reflected in the physiological stress response displayed. These fine-tuned changes in the endocrine system suggest that predation affects the phenotypic plasticity of adult rabbits.

**Acknowledgments** We are very grateful to J.C. Rivilla, J. Román, and several students, C. Estabilllo, J.M. Clavijo, and J. Martínez for their help with the field work and Edith Klobetz-Rassam for assistance on the analysis of the fecal samples. Heiko G. Rödel and Eloy Revilla gave valuable comments on earlier drafts of the manuscript. Jörg Ganzhorn and two anonymous reviewers greatly improved the manuscript. This research was partially funded by the projects CGL2004-00346/BOS of the Spanish Ministry of Education and Science and the Caixa Foundation. The Consejería de Medio Ambiente Junta de Andalucía built the rabbit enclosures under the LIFE 02NAT/8609 conservation program. Land Rover España S.A. provided the four-wheel vehicles used during this study. Z. Tablado was supported by a FPU scholarship from the Spanish Ministry of Education.

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