ORIGINAL ARTICLE

Stressful living in lower-quality habitats? Body mass, feeding behaviour and

physiological stress levels in wild wood mouse populations

Running head: Mice reactions to habitat quality

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Abstract

Wild populations are continuously subjected to changes in environmental factors that pose different challenges. Body condition and hormones have been commonly used as health indicators due to their potential correlation with fitness. In the present study, we analyzed whether habitats of different quality, influenced body mass, food intake and physiological stress levels in wild wood mice (*Apodemus sylvaticus*). Field work was seasonally carried out in Holm oak woods and pine forests in central Spain. 93 wood mice from 4 different populations (2 per habitat type) were live-trapped. From each captured individual we noted body mass and food intake, measured as the amount of bait remaining in each trap. The physiological stress levels were measured non-invasively in collected fresh faeces by quantifying faecal corticosterone metabolites (FCM) with a 5α-pregnane-3ß,11ß, 21-triol-20-one enzyme immunoassay. Wood mice abundances decreased from spring to summer, were higher in Holm oak woods than in pine forests and also resulted in different age-class distribution between both habitats.

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Individuals inhabiting pine forests showed a lower body mass and increased food intake probably because the comparatively lower food quality and availability in this habitat. Further, these individuals showed increased physiological stress levels, likely due to the lower quality habitat regarding both food and vegetation cover availability. Overall, besides affecting local wood mouse abundance, our study underscores the effect of habitat quality on body mass, food intake and the endocrine stress response. Considering wood mouse pivotal position in ecosystems, these results could help in the understanding of environmental traits hampering viability of wild populations.

Key-words: *Apodemus sylvaticus*; body condition; corticosterone, environmental factors; faecal corticosterone metabolites; feeding behaviour

Introduction

Habitat structure, as the physical arrangement of objects, is a key ecological factor influencing environmental conditions and, in consequence, wildlife populations (Bell *et al*. 2012). Further, both the quality and the availability of resources (e.g. food and shelter) influence habitat quality, and consequentially affecting animals´ well-being, reproduction and survival, i.e. on animal´s fitness (McLoughlin *et al*. 2006; Johnson 2007; Mortelliti *et al*. 2010). There is increasing evidence supporting that differences among habitats, due to variation in habitat quality, play an essential role in determining individuals' distribution as well as regulating temporal and spatial population dynamics (Pulliam and Danielson 1991; Lin and Batzli 2001; Jaquiéry *et al*. 2008; Janova *et al*. 2011; Navarro-Castilla *et al*. 2014a). Equally, variation in habitat due to for example climatic or seasonal changes also conditions food availability and population densities due to shifts in environmental features (Fernández *et al*. 1996; Ouin *et al*. 2000; Díaz *et al*. 2010; Janova and Heroldova 2016; Sunyer *et al*. 2016).

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Generally, habitat quality involves a complex combination of physical, chemical and biological elements and varies with factors such as quality of food, competition, population density and risk of predation. Small mammal species highly rely on microhabitat attributes such as vegetation complexity and soil characteristics for habitat preference and selection (Ellis *et al*. 1997; Sheffield *et al*. 2001; Holland and Bennett 2007; Navarro-Castilla *et al*. 2017a), likely because their important value for food, safely foraging and building nests or refuges as protection against predators (Newman *et al*. 1988; Jacob 2008; Rosalino *et al*. 2011a). Increased habitat quality is expected to increase habitat attractiveness, therefore, an increased number of individuals should be able to exploit a better quality habitat. Consequently, increased densities can turn resources limited for the population, leading also to a higher intraspecific competition and substantially diminishing the overall value of the habitat (i.e. decreasing habitat's quality perception). Because of the increasing densities, especially in the breeding season and during seasons when resources are most limited, agonistic interactions among conspecifics are expected to increase (Malo *et al*. 2013). As a consequence, individuals might spread among habitats which vary in habitat quality. Thus, dominant individuals are expected to occupy the highest quality habitats while subordinate and juveniles are pushed to occupy an expanding range of lower-quality habitats (Gurnell 1978; Dobson 1982; Malo *et al*. 2013; Martineau *et al*. 2016). These source-sink dynamics driven by competition may lead to a higher population density in the lower-quality habitat (Van Horne 1982; Martineau *et al*. 2016). Therefore, density could be a misleading indicator of habitat quality (Van Horne 1983) and consequently, focusing on other individual attributes is required. In this regard, body condition and physiological parameters have been proved to be good indicators for properly evaluating seasonal changes, both habitat availability and quality, as well as threats of landscape

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modifications in wildlife (Díaz and Alonso 2003; Romero 2004; Homyack 2010; Janin *et al*. 2011; Ebensperger *et al*. 2013; Ditmer *et al*. 2015).

Among physiological metrics, measurement of glucocorticoids (cortisol or corticosterone depending on the species) has been utilized as a suitable tool for evaluating both the endocrine status and responses to stressful stimuli in different animal species (Sheriff *et al*. 2011; Navarro-Castilla *et al*. 2014ab; Shutt *et al*. 2014; Casas *et al*. 2016; Dantzer *et al*. 2016; Fauteux *et al*. 2017; Sánchez-González *et al*. 2018). Environmental factors (e.g. food availability, invasive species, competition for resources or predation) have been reported to be perceived as potential stressors for wildlife populations (Kitaysky *et al*. 1999; Frid and Dill 2002; Clinchy *et al*. 2013; Creel *et al*. 2013; Graham *et al*. 2017). Wildlife species and individuals within a species can respond in different ways to fluctuating or adverse changes in habitat quality, i.e. some species/individuals have little or no response to a stressor that evokes a large response in others (Cockrem 2013). Several investigations have addressed the potential effect of lower quality habitats on the physiological stress-related hormone levels in different wild animal populations (e.g. fishes: King *et al*. 2016; amphibians: Homan *et al*. 2003; reptiles: Cash and Holberton 2005; birds: Zhang *et al*. 2011; and mammals: Escribano-Ávila *et al*. 2013). However, few works have been done to study the physiological stress effects of habitat traits on small mammals (Hik *et al*. 2001; Rogovin *et al*. 2006; Sheriff *et al*. 2012; Rehnus *et al*. 2014).

The wood mouse is a generalist species (Montgomery and Gurnell 1985) commonly found in most of wildlife rodent communities (Tellería *et al*. 1991; Janova and Heroldova 2016). Despite its adaptability to cope and forage in a great variety of habitats (Rodríguez and Peris 2007; Janova *et al*. 2011; Ruiz-Capillas *et al*. 2013), we have previously reported how the wood mouse and other rodent species inhabiting in

disturbed habitats showed increased physiological stress baseline levels (Navarro-Castilla *et al*. 2014ab). Although numerous studies have reported that wood mouse is not uniformly successful in all habitats because habitat quality affects its demography, diet, space use and phenotypic characteristics (Unnsteinsdottir and Hersteinsson 2011, see also references cited above), to the best of our knowledge, little is known about the effects of habitat quality on the physiological stress levels of wild wood mouse populations.

Due to this lack of information, in the present study we aimed to examine whether differences in overall habitat quality influenced: 1) abundance, 2) body mass, 3) food intake and 4) physiological stress levels in the wood mouse (*Apodemus sylvaticus*). In the study area, comparing pine forests and Holm oak woods, these last support a higher tree density as well as increased vegetation cover and height which are important as protection from predators. Increased miscellaneous vegetation availability also provides greater food opportunities for a species like the wood mouse that has a wide food spectrum including seeds, grains, vegetative parts of plants and invertebrates (Khames and Aulagnier 2007; Butet and Delettre 2011). Further, the wood mouse heavily relies on acorns and pinecones as part of its diet (Muñoz *et al*. 2009; Manso *et al*. 2014) when mature acorns and pinecones start to fall naturally on September-October and November, respectively (Gea-Izquierdo *et al*. 2006; Calama *et al*. 2011). Despite seed production can vary between years, overall, annual seed production is higher in Holm oaks woodlands (5-12 kg per tree; Gea-Izquierdo *et al*. 2006) compared to pine forests (1.4-2.6 kg per tree; Calama *et al*. 2011). Thus, based on the lower availability of both food resources and vegetation refuges in pine forests, we hypothesized that in this habitat: 1) Wood mice abundance will be lower, 2) Individuals will present a lower body mass, 3) Food intake inside traps is expected to be higher, and

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4) Physiological stress baseline levels will be elevated, consistent with an increase in stress in poorer habitats. Further, all these responses are expected to be seasonally influenced being especially noticeable in summer, i.e. during the limiting season.

Material and Methods

Study area

The study was conducted in the Valdelatas public domain forest (Madrid, 40°32′N 3°41′W; Figure 1). With an extension of ca. 259 ha, the study area consists of dense Holm oak forests (*Quercus ilex ballota*) interspersed with pine tree reforestations (*Pinus pinea* and *Pinus pinaster*). This distribution of pine tree plantations surrounded by Holm oak forests allowed us to select distant (ca. 500 m) and thus, independent, Holm oak and pine tree plots. The study area is within the Mediterranean climatic region according to the updated Köppen-Geiger climate classification (Kottek *et al*. 2006). Climatic conditions are the same for both types of habitat, but they significantly differ in their associated vegetation. Tree density is higher in Holm oak woods where it is also present an abundant and dense scrubland of gum rockrose (*Cistus ladanifer*), while understory in pine forests is restricted to an open grassland matrix with reduced and scattered shrub cover.

Live trapping and data collection

Wood mice were live-trapped with Shermann® traps. Five trapping sessions were carried out in both habitats during one year: spring-April, summer-July and autumn-October (2009); winter-February and spring-April (2010). Of each habitat type, we trapped in different forests by selecting two independent sampling plots (Figure 1). Mean distance between selected plots was on average 500 m, which is larger than the ranges of the target species in different habitats (Corp *et al.* 1997; Rosalino *et al*. 2011b). Therefore, wood mice captured in each plot belonged to a different population

constituting independent replicates for mice captures both between forests of the same habitat and between habitats. This is also supported because mice from one plot were not recaptured in the other plots. Besides, taking into account wood mice movement patterns and distance travelled per night (<100 m in the same direction, Corp *et al*. 1997) as well as the mean home range size of the wood mouse in woodlands (1.124 ± 0.925 ha, Rosalino *et al*. 2011b), sampling plots were at least 100 m apart from the habitat edge in order to avoid mice migration movements between each selected plot and the adjacent habitat. In each plot we set 25 traps/area in a 5x5 grid (7 m of separation between traps). Traps were baited with toasted corn (4 g) and hidden under vegetation in order to protect captures from both predators and weather conditions. Sherman traps were operated during five consecutive nights, with a total effort of 2500 traps-night. Traps were set at dusk and checked in the morning (mean time opened 10h 6´).

Individuals captured were identified to species. Sex and breeding status were determined for wood mice according to Gurnell and Flowerdew (2006). Briefly, the anal-genital distance is larger in males and regarding breeding status, in males testicles are enlarged and descended into the scrotal sac while females present noticeable abdominal and thoracic nipples as well as the vaginal membrane perforated. Wood mice captured were quickly handled and released in the point of capture. In order to identify recaptures, wood mice were marked with harmless paints (red food colouring: Ponceau-4R E124).

Body mass and food intake

Each wood mouse captured was weighted with a 100 g hand-held scale and age class was estimated following Navarro-Castilla and Barja (2014a): juveniles (< 13 g), subadults (13-<20 g) and adults (≥ 20 g). An electronic balance (Giros PG-500;

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precision 0.01 g) was used to weigh the 4 g of roasted corn provided inside each trap and the amount of bait that was not consumed, so determining food intake inside trap by each individual. Later, to have a more precise measurement of food intake this value was accordingly corrected (divided by the body mass of each individual).

Faecal corticosterone metabolites (FCM)

Fresh faeces from each wood mouse captured in a Sherman trap were collected only during the early morning checking (i.e. 7:00–9:00 am) to minimize the effect of environmental conditions and microbial degradation (Millspaugh and Washburn 2003; Möstl *et al*. 2005; Barja *et al*. 2012). This way, we also avoided the influence of circadian rhythm in excretion patterns (Touma *et al*. 2003; Touma *et al*. 2004). Navarro-Castilla et al. (2017b) recently showed that wood mice were trapped on average 6 h after trap activation at dusk. Thus, since traps were opened ca.10 h, individuals spent about 4-5 h inside traps. In addition, FCM peak concentrations have been observed in wood mice faeces between 8-12 h after the ACTH injection (Navarro-Castilla et al. 2017b). Hence, we conservatively assumed that trapping was not likely influencing the physiological stress response since captured mice spent less than 8h inside traps. Whenever urine was detected in the trap, faecal samples were rejected in order to avoid cross contamination. Collected faecal samples were stored at -20º C until analysis.

FCM were extracted from the faeces following Touma *et al*. (2003) and Palme *et al*. (2013). Frozen faecal samples were dried and homogenized, and then 0.05 g was mixed in 1 ml of methanol (80%) in an eppendorf tube. Following, tubes were shaken on a multivortex (30 min) and then centrifuged at 2500 *g* (15 min). Supernatants were diluted with assay buffer (1:10) and stored at -20ºC until analysis. FCM were quantified by an established 5α-pregnane-3ß,11ß,21-triol-20-one enzyme immunoassay (EIA) measuring metabolites with a 5α-3ß,11ß diol structure (Touma *et al*. 2003). This EIA

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has been previously used for monitoring FCM concentrations in wood mice (Navarro-Castilla and Barja 2014b; Navarro-Castilla *et al*. 2014b).

Statistical analyses

Preliminary analyses showed that mean values from all the variables studied (wood mice abundance, body mass, food intake and FCM levels) were related to habitat type being similar in forests within the same habitat, so samples from both plots of each habitat were analyzed together. Differences in the number of captures due to habitat and season were analyzed by Chi-square tests (χ^2). Since sex ratio was equally male-biased along habitats and seasons, and there were no active breeding individuals in the analyzed sample, both sex and breeding status were not included in the statistical analyses. Body mass of individuals (response variable) was analyzed by a General Linear Model (GLM) where habitat type (pine forest / Holm oak woods), season (spring / summer) and recapture (new capture / recapture) were included as fixed factors and food intake as covariate to control the effect of the bait consumed. Similarly, a GLM was done to analyze food intake (corrected by body mass) in relation to habitat, season and recapture. Finally, we performed a GLM for analyzing differences in FCM levels (variable response) due to habitat, season, recapture and food intake (corrected for body mass).

Results were considered significant at α <0.05. Data are presented as mean ± standard error (SE). We used SPSS 22.0 software (SPSS Inc, Chicago, IL, USA) for the statistical analyses.

Results

During the study period we had a total of 201 wood mice captures. However, the absence of wood mice captures in pine forests from autumn 2009 to spring 2010, and the small sample size of adults captured in pine forests in spring and summer 2009

(Holm oak woods: 68; pine forests: 2) did not permit comparative statistical analysis between habitats. Therefore, only data from juveniles and subadults captured in spring and summer 2009 were considered further. The number of captures was significantly higher during spring (91.4%) compared to summer (8.6%) (χ^2= 63.753, df = 1, $P <$ 0.0001, n = 93). Wood mice were more frequently captured in Holm oak woods (71%) than in pine forests (29%) (χ^2= 16.355, df = 1, $P <$ 0.0001, n = 93). Further, age-class population distributions significantly differed in pine forests (juveniles: 18, subadults: 9; χ^2= 11.111, df = 1, $P <$ 0.0001) and in Holm oak woods (juveniles: 10, subadults: 56; χ^2= 48.576, df = 1, $P <$ 0.0001), showing and inverse pattern between both habitats (Figure 2).

Body mass and food intake

Neither food intake nor recapture influenced body mass of captured individuals (Table 1A). Individuals captured during spring showed a higher body mass (16.31 ± 0.32 g) than wood mice captured in summer (14.50 ± 1.43 g), however, results from the GLM showed that body mass of individuals was just significantly explained by habitat type (Table 1A). Thus, individuals inhabiting in pine forests showed a lower body mass (13.30 ± 0.64 g) than those from the Holm oak woods (17.32 ± 0.26 g). Separate analyses revealed that differences in body mass due to habitat type were significant for juveniles (pine forests: 11.35 ± 0.48 g, Holm oak woods: 12.60 ± 0.37 g; $F_{1,27}$ =10.271, P = 0.004) but not for subadults (pine forests: 17.21 ± 0.40 g, Holm oak woods: 17.98 ± 0.19 g; $F_{1,64}$ =2.448, P = 0.123).

Regarding food intake (corrected by body mass), recaptures did not resulted as a significant factor. Wood mice consumed more bait in summer (0.23 ± 0.04 g) than in spring (0.17 ± 0.01 g) but differences were not statistically significant (Table 1B). Food intake was higher in pine forest (0.22 ± 0.03 g) than in Holm oak woods (0.16 ± 0.01 g),

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and the interaction between habitat*season showed how this difference was greater in summer (Table 1B, Figure 3).

FCM levels

The statistical model showed that the only significant factor explaining the variation found in FCM levels was the type of habitat (Table 2, Figure 4). Wood mice inhabiting in pine forests showed higher FCM levels (2152 ± 309 ng/g dry faeces) than those individuals captured in Holm oak woods (976 ± 104 ng/g dry faeces). Neither season, recapture nor food intake significantly influenced FCM. Interactions between factors were not statistically significant (Table 2).

Discussion

In the present study wood mouse abundances were higher in spring compared to summer, these results are in accordance with previous works (Rosário and Mathias 2004; Amori *et al*. 2015; Gasperini *et al*. 2016; Sunyer *et al*. 2016). A plausible explanation for the higher abundances in spring would be based on the reproductive cycle since, in the Mediterranean region, it occurs from autumn to spring with a breeding pause in summer (Fons and Saint-Girons 1993; Torre *et al*. 2002). Further, wood mouse population sizes tend to decrease during summer periods because the adverse climatic conditions of the Mediterranean region (mainly drought) significantly reduce the overall availability of food resources being a main cause of mortality (Torre *et al*. 2002; Díaz *et al*. 2010; Sunyer *et al*. 2016). Regarding habitat type, a large number of individuals inhabited Holm oak woods while few captures were obtained in pine forests. Similar differences due to habitat type have been previously found in the same and other rodent species (Unnsteinsdottir and Hersteinsson 2011; Malo *et al*. 2013; Bogdziewicz and Zwolak 2014; Barja *et al*. 2016; Gasperini *et al*. 2016). Rodent species highly rely on plant cover and food resources availability (Tarjuelo *et al*. 2011;

Arnan *et al*. 2014; Noguerales *et al*. 2015) and the higher food quality generally leads to

higher rodent densities (Janova et al. 2016). Thus, the low wood mice abundance in pine

forest may indicate a lower-quality habitat due to the lesser availability of food

resources and the limited vegetation cover as protection against predators. Similarly, the

scarcity of resources may consequently lead to larger home ranges in the wood mouse

(Gorman *et al*. 1993; Corp *et al*. 1997) while increased food availability may result in

reduced home ranges (Akbar and Gorman 1996) and increased reproductive output

since female wood mice advance reproduction (i.e. breed earlier) and could have larger

litter sizes (Díaz and Alonso 2003). Therefore, increased home ranges and lower

reproduction rates may also explain the lower wood mouse abundance in pine forests.

Further, we also found an interesting inverse relation in age-class distribution among

both habitats supporting the hypothesized differences in habitat quality. While subadults

were widely captured in Holm oak woods, the youngest individuals seem to be expelled

to pine forests. This variation in age population dynamics with habitat type has been

previously found in this and other rodent species (Gorman *et al*. 1993; Meri *et al*. 2008;

Malo *et al*. 2013; Martineau *et al*. 2016). Since the value of habitat may be reduced by

interferences from dominant individuals (Morris 2003), it seems that interspecific

competition, territoriality and the higher aggression rates by largest individuals

(Hernández et al. 2018) may be responsible for this habitat segregation.

Body mass and food intake

Vegetation and food availability are highly influenced by seasonal changes. However,

despite increased vegetation and overall trophic resources are associated to rainy

periods (Díaz *et al*. 2010), we found a non-significant weight decrease in summer.

Probably, individuals may have occupied the home ranges vacated during the

population decline in summer as it has been seen in other rodent species (Halama and

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Dueser 1994). This way, the negative effects of the limited season (summer) could be compensated by the reduced abundances and competition for resources. Regarding habitat type, overall, body mass was higher in individuals captured in Holm oak woods compared to those from pine forests. Besides increased availability of food resources, Holm oak woods from the study area also provided increased vegetation cover as protection from predation risk, thereby improving the effective foraging area and time required (Gorman *et al*. 1993; Tew *et al*. 2000). So, the lower body mass of wood mice inhabiting pine forests could be a further indicator of living in a lower quality habitat (Unnsteinsdottir and Hersteinsson 2011). Alternatively, since weaker and submissive individuals are generally pushed to the lower quality habitats (Malo *et al*. 2013; Martineau *et al*. 2016), the overall lower body mass found in those individuals from pine forests could be also partially explained as the consequence of the weaker body condition of individuals entering into this lower-quality habitat. However, differences in body mass between habitats were statistically significant for juveniles but not for subadults. Considering the increased wood mice abundance in Holm oak woods then, interspecific competition was expected to be higher with dominant individuals (i.e. adults) interfering with exploitation choices of subordinates (both juveniles and subadults). But the higher body mass of wood mice in this habitat, especially in juveniles, suggest that Holm oak woods may provide adequate food supply allowing all individuals to forage with profit and a faster growth. However, in pine forests subadults were less constrained by adults (only two adults were captured in this habitat) so they were able to freely forage as dominant individuals, which may have minimized differences in body mass of subadults between both habitats.

The overall lower body mass found in pine forests was supported by the increased food intake of wood mice in this habitat compared to the Holm oak woods. A recent study

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revealed that food intake was not conditioned by the time that each wood mouse was trapped (Navarro-Castilla *et al*. 2017b), thus, the differences in food intake between habitats cannot be attributable to a different duration inside traps. The higher food intake in pine forests could be also consequence of the lack of food resources in this habitat and wood mice would maximize each food source found (as bait inside traps) to meet their energy requirements. This will be in accordance with results from Monarca *et al*. (2015), who under laboratory conditions observed that wood mice increased food intake after several periods of food starvation.

FCM levels

In the wood mouse, as in other rodent species, higher FCM levels are associated with the breeding season (Navarro-Castilla and Barja 2014b; Navarro-Castilla *et al*. 2014b). Considering that the studied individuals were captured in spring (end of the breeding season) and summer (breeding pause), and beside, none of the individuals analysed was actively breeding, this would explain why we did not found season as a significant factor explaining variation in FCM levels. Habitat type was the only factor with a significant influence on the physiological stress response. We have recently found that vegetation and soil characteristics (e.g. reduced cover and higher soil compaction) were related to increased FCM levels in the Algerian mouse, *Mus spretus* (Navarro-Castilla *et al*. 2017a). In addition, food depreviation triggers a physiological response involving the long-term release of energy-mobilizing glucocorticoids like corticosterone (Kitaysky *et al*. 2001; Wingfield and Romero 2001). Thus, the higher FCM levels found in pine forests are likely the result of habitat traits leading to depleted food to cover energy demand. In other words, individuals may be modulating their stress response to better cope with this specific habitat. Supporting this suboptimal environment hypothesis, several studies have reported that differences in GC levels may reflect local variation in

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habitat quality and predator environments (Hik *et al*. 2001; Mateo 2006; Mateo 2007; Monclús *et al*. 2009; Bauer *et al*. 2013). Since baseline GC concentrations are often used as an index of an animal's health or stress level (Bonier *et al*. 2009), increased FCM levels found in wood mice inhabiting pine forests could have long-term adverse effects on health, fertility and survival as has been suggested for other mammal species (Möstl and Palme 2002; Romero 2004; Charbonnel *et al*. 2007; Martínez-Mota *et al*. 2007).

In summary, although the wood mouse is considered as a flexible species able to exploit a wide range of environments, we have shown that wood mice can be influenced by habitat type which ruled population dynamics, age-class distributions, body mass and feeding behaviour. Our results also demonstrated that the endocrine stress response also differed between habitat types, being then a non-invasive and valuable biomarker of habitat quality. However, seasonal effects on the variables studied seem to be diluted or compensated by the reduced wood mouse abundance in summer (because the natural population decline). Our study represented only a brief period (i.e. spring and summer) in the annual history of both habitats, therefore, long-term spatial and temporal comparison studies are required to better understand the complex trade-off in quality and quantity of resources between habitat types, seasonal variation and the inherent individual traits (e.g. age, social rank and body condition) influencing the specific responses of wild wood mice populations to environmental challenges through the year. Further study on the behavioural and physiological ecology of the wood mouse may also help understanding to monitor, detect and even prevent conservation problems in other more sensitive wild species.

Ethical approval

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All applicable institutional and/or national guidelines for the care and use of animals were followed. Manipulations of animals were done under the permit of the Comunidad Autónoma de Madrid (Spain) reference number 10/422509. Further, in this study we closely followed ethical standards of the Universidad Autónoma de Madrid and all regulations concerning handling and treatment of animals in accordance with the European Communities Council Directives of 24 November 1986 (86/609/EEC) for animal experiments.

Acknowledgements

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Accepted Article

Wingfield, J.C., Romero, L.M. (2001). Adrenocortical responses to stress and their modulation in free-living vertebrates. Comprehensive Physiology 211-234.

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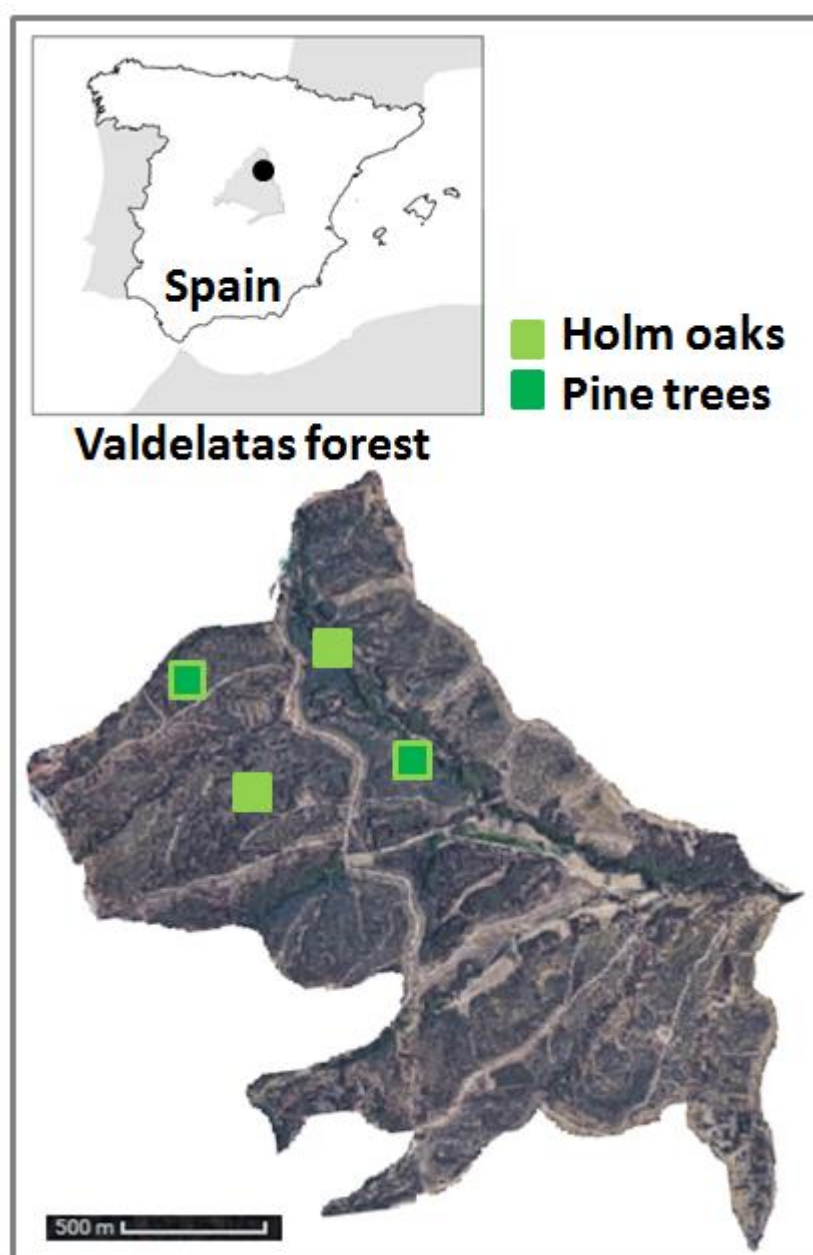
Figure Legends

Figure 1 Study area and distribution of sampling plots in Holm oak woods and pine
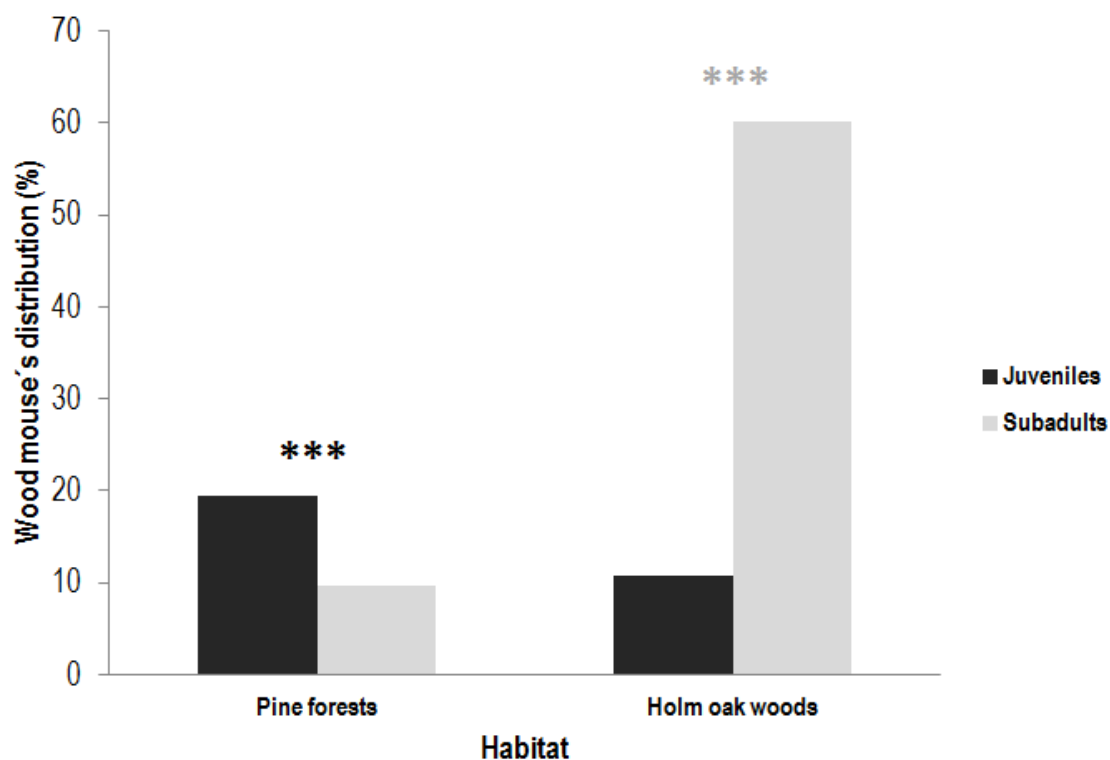
forests.

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Figure 2 Percentage of wood mice captured in relation to habitat and age-class of individuals. Asterisks indicate significant differences (***p < 0.001) between age groups within the same habitat, pine forests (black asterisks) and Holm oak woods (gray asterisks).
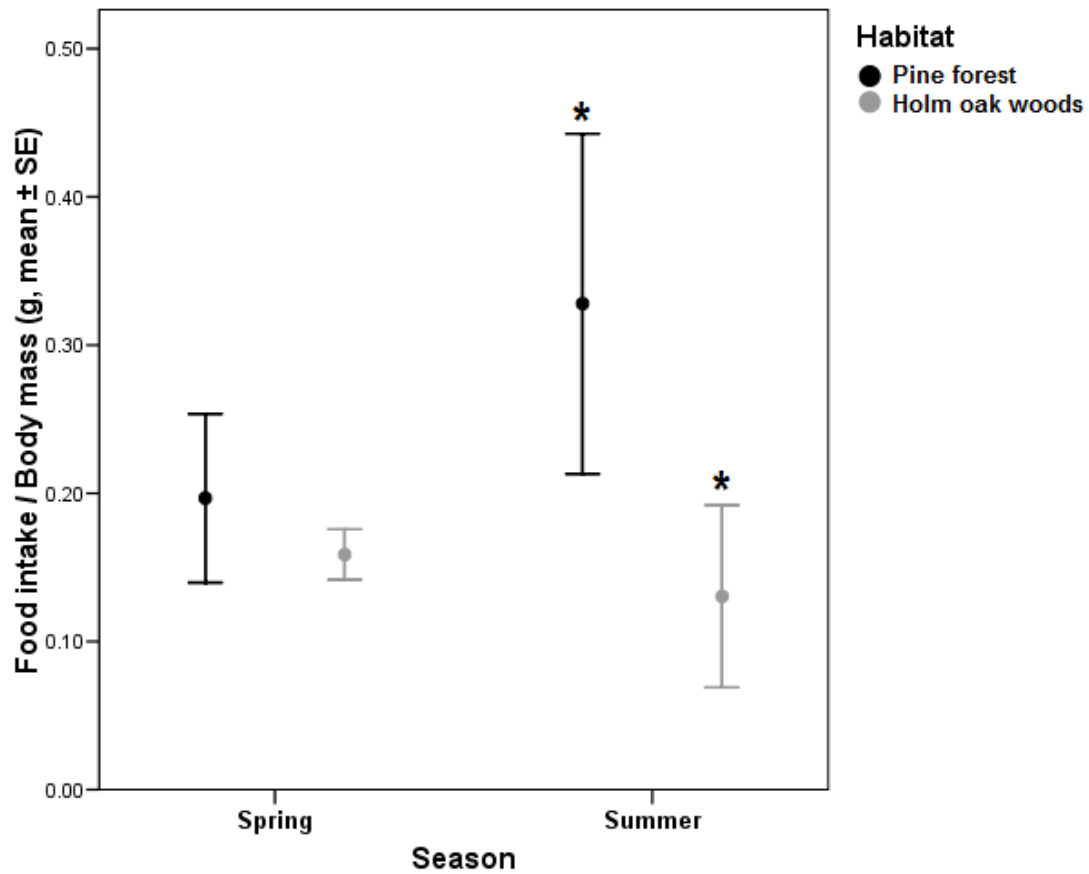
Figure 3 Food intake-corrected by body mass (g, mean ± SE) in relation to habitat and season. Asterisks indicate significant differences (*p<0.05) between habitats within the same season.
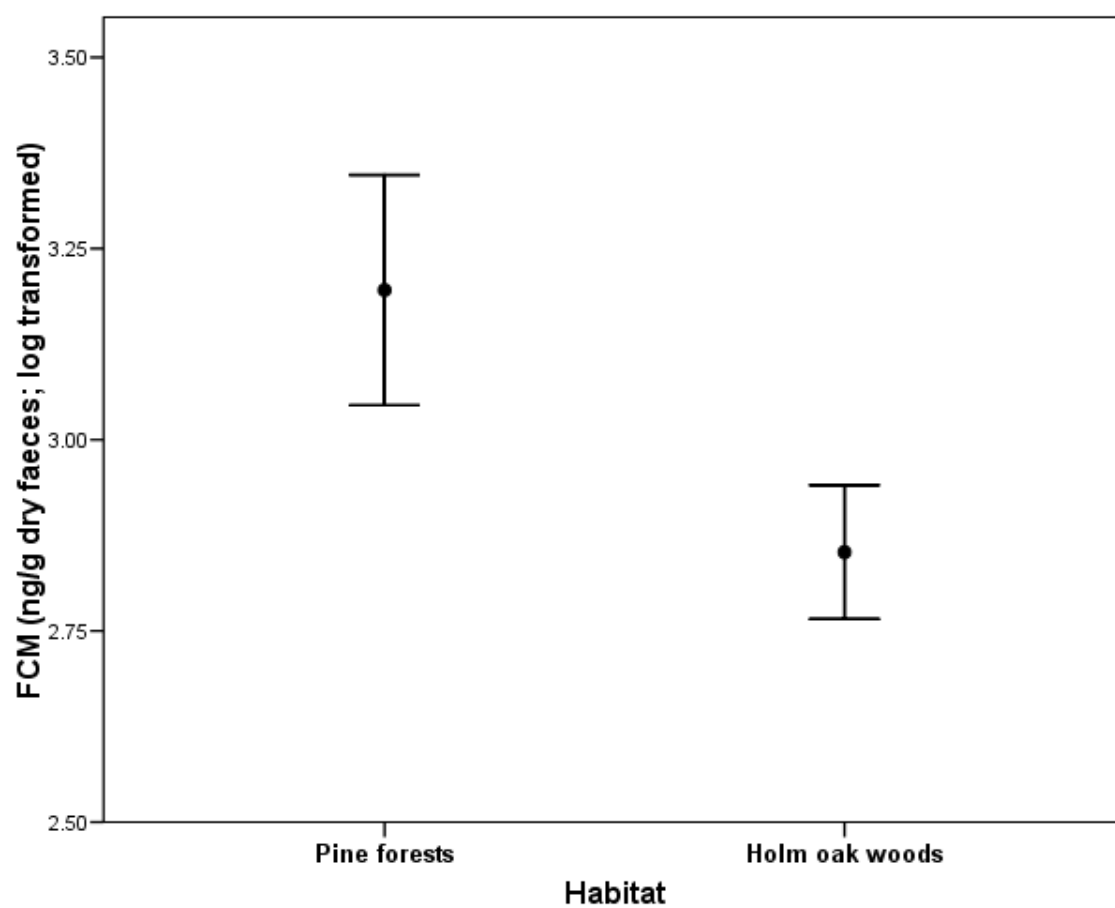
Figure 4 Log-transformed FCM levels (ng/g dry faeces; mean ± SE) according to

habitat type.

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Table 1 Results from the GLMs analyzing body mass (A) and food intake-corrected by

body mass (B) in relation to habitat, season and recapture.

A. Body mass

Variables	df	*F*	*P*
Habitat (pine / oak)	1	27.741	<0.001
Season (spring / summer)	1	1.762	0.188
Food intake	1	1.568	0.214
Recapture (new / recapture)	1	0.001	0.971
Habitat*Season	1	2.506	0.117
Habitat*Recapture	1	0.263	0.609
Season*Recapture	1	2.448	0.121
Habitat*Season*Recapture	1	1.249	0.267
Error	84		

B. Food intake (corrected by body mass)

Variables	df	*F*	*P*
Habitat (pine / oak)	1	11.790	0.001
Season (spring / summer)	1	2.883	0.093
Recapture (new / recapture)	1	0.000	0.993
Habitat*Season	1	6.675	0.011
Habitat*Recapture	1	0.014	0.907
Season*Recapture	1	0.528	0.470
Habitat*Season*Recapture	1	1.056	0.307
Error	85		

Table 2 Results of the GLM testing the effect of environmental and individual factors

on faecal corticosterone metabolites in the wood mouse.

Variables	df	*F*	*P*
Habitat (pine / oak)	1	6.393	0.013
Season (spring / summer)	1	0.760	0.386
Food intake (corrected by body mass)	1	0.057	0.812
Recapture (new / recapture)	1	0.002	0.968
Habitat*Season	1	0.076	0.783
Habitat*Recapture	1	0.809	0.371
Season*Recapture	1	0.526	0.470
Habitat*Season*Recapture	1	0.002	0.962
Error	84		