



# When food access is challenging: evidence of wood mice ability to balance energy budget under predation risk and physiological stress reactions

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## Abstract

Prey species must balance the energetic cost of locomotion, foraging effort, and predation risk. In our work, we assessed how the wood mice (*Apodemus sylvaticus*) balance these costs by manipulating food access difficulty and predation risk. Live trapping was conducted in Madrid (Spain) where 80 Sherman traps were set in four different plots. To assess how wood mice manage their energy budget, all traps were first subjected to a control period followed by four treatments of 3-day duration in which food access was experimentally manipulated (free access, straw balls, straw balls wrapped in metal wire, opened plastic bottles, closed bottles). Predation risk was simulated by exposing half of the traps to fox feces. To quantify food intake, we weighed the remnants of food left by each captured mouse. Furthermore, we collected mouse fecal samples from traps to evaluate the physiological stress response by quantifying fecal corticosterone metabolites (FCM). Results showed that despite mice generally avoiding traps treated with fox feces, predation risk did not modulate food intake or FCM levels. By contrast, the experimental manipulation of food access determined the amount eaten and increased FCM levels, probably owing to the different degrees of difficulty and the energy required to obtain the food. Moreover, recaptured individuals ate more, indicating that experience critically determines mice ability to reduce the costs of accessing food. By analyzing the joint variation between mice intake and FCM levels depending on food restriction treatments, we found that mice were able to modulate their energetic expenditure depending on food access difficulty probably in order to achieve a positive marginal value of energy.

## Significance statement

For wild animals, to optimally balance the energetic budget is critical to maximize fitness. However, foraging decisions are influenced by numerous internal and external factors which are poorly understood. We studied free-ranging mice behavioral and physiological stress response to different food restriction treatments, testing also the influence of predation risk and experience. We found that predation risk modulated mice capturability but not the feeding behavior or the stress response. We also discovered that mice seem to be able to adjust energy allocation when a novel resource appear, and that energy budget optimization may be mediated via glucocorticoid release and individuals experience.

**Keywords** Food access difficulty · Food restriction · Risk of predation · Small mammals · Glucocorticoids

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## Introduction

The process of obtaining food has some inherent long- and short-term costs and animals must adaptively balance their energy intake and utilization (Dill 1987; Mangel and Clark 1988; Lima and Dill 1990; van Gils and Tijssen 2007). Searching and obtaining food is inevitably connected to the productivity of the individual (i.e., growth rate, reproduction, and lactation) and the trade-off may be controlled by the amount and quality of food consumed (Cruz-Rivera and Hay 2000; Morris and Davidson 2000). Consequently, an increase in the effort spent in obtaining food can lead to a decrease in the future survival probability as a result of energy demands exceeding energy intake. On the other hand, restricted food access has extensive effects on the behavior and physical condition of animals (Blum et al. 1985; Hamilton and Bronson 1985; Appleby and Lawrence 1987; Voltura and Wunder 1998; Le Galliard et al. 2004). Therefore, properly balancing the energy budget should be an important selective force for the evolution of life-history traits.

In natural environments, changes in nutrient availability and thus in diet characteristics are rather common, testing the individual's plasticity (Cresswell 2003). In this regard, behavioral responses are important for animals to persist in an ever-changing environment (Beale and Monaghan 2004; Sih 2013; Polo-Cavia and Gomez-Mestre 2014). Furthermore, prey species dynamically adjust their behavior in response to perceived predation risk in order to reduce the probability of being preyed upon (Lima and Dill 1990; Brown and Kotler 2004). This behavioral flexibility is especially crucial in habitats where predation risk is spatially and temporally variable (Houston et al. 1993; Werner and Anholt 1993; Agrawal 2001). Antipredatory responses redirect time and energy from other fitness-enhancing activities, such as food seeking and reproduction (Frid and Dill 2002). Predation risk has been demonstrated to affect the food intake and body mass of rodents (Morley and Levine 1982; Krahn et al. 1990; Barreto and Macdonald 1999; Navarro-Castilla et al. 2014a, b; Monarca et al. 2015; Sánchez-González et al. 2017). Therefore, individuals have to make decisions based on environmental predation risk cues (including visual and olfactory stimuli), evaluating the costs and advantages of each possible option and hence only performing antipredator responses if the benefits counterbalance the cost (McNamara and Houston 1987; Lima and Dill 1990; Lind and Cresswell 2005). Since time allocated to food handling can be modulated by predation risk (Lima and Valone 1986; Newman et al. 1988), the effort required and expended for food access could be influenced by predator cues in the same way (Verdolin 2006; Hernández et al. 2019). Though some studies have examined the amount of food eaten by prey under predation risk (Díaz 1992; Epple et al. 1993; Barreto

and Macdonald 1999; Navarro-Castilla and Barja 2014a, b; Monarca et al. 2015; Sánchez-González et al. 2017), to the best of our knowledge, none of them has evaluated the implications of variation in food access difficulty combined with predation risk.

Besides conditioning animal behaviors, environmental conditions may also impact the physiological stress response profile of the individuals (Kitaysky et al. 1999; Schoech et al. 2004; Levay et al. 2010; Navarro-Castilla and Barja 2019). When a stressful stimulus is perceived, the hypothalamic pituitary adrenocortical (HPA) axis is activated, increasing the concentrations of catecholamines and glucocorticoids (GC) in the bloodstream in order to mobilize the reserves needed to cope with acute environmental demands (Romero 2004; Bonier et al. 2009). Even though short-term activation of this system is an adaptive response that increases individual's energy availability to cope with immediate challenges (Wingfield et al. 1998), chronically elevated GC levels can be deleterious, leading to immune system suppression, gastrointestinal ulcerations, reproductive failure, and endocrine disruption (Stewart 2003; Romero 2004). In the wood mouse, habitat quality (Navarro-Castilla and Barja 2019) and additional stressors, such as predation risk (Sánchez-González et al. 2018b), can cause physiological changes. However, the physiological response to the difficulty of accessing food under the threat of predation risk does not seem to have been previously evaluated in this species.

The aim of this study was to analyze how food intake and physiological stress responses vary depending on food access difficulty and predation risk in the wood mouse (*Apodemus sylvaticus*). We predicted that food intake would decrease with the increasing food access difficulty, due to the material restriction itself and the associated energetic cost of accessing the food. Furthermore, under predation risk, we predicted mice to decrease food intake as a fear response, especially in the most difficult access treatments, because mice were expected to allocate more time in antipredator responses (freezing, try to hide, attempt to flight by trying to escape from the traps, and so forth) than on obtaining nourishment. We also predicted a higher food intake in recaptured mice as a consequence of the previous experience. As for the physiological stress response, we expected higher FCM levels in mice facing the most difficult food access treatments due to a higher effort made, and particularly in those individuals exposed to predation risk since this threat was expected to turn the situation even more stressful. Furthermore, we also considered the effect of individual factors such as sex and breeding condition since they have been previously demonstrated to influence both behavioral and physiological responses in this rodent species (Navarro-Castilla and Barja 2014a, b; Sánchez-González et al. 2017, 2018b; Hernández et al. 2018b).

## Materials and methods

### Study area

Fieldwork was carried out in “Monte de Valdelatas” (Madrid, Spain), a Mediterranean forest situated at an altitude of 650 m a.s.l. The area contains forests of holm oak (*Quercus ilex ballota*) and scrubland mainly composed of gum rock roses (*Cistus ladanifer*), thyme (*Thymus zygis*), and umbel-flowered sun roses (*Halimium umbellatum*). Predators such as red foxes (*Vulpes vulpes*) and common genet (*Genetta genetta*) inhabit this area (Monclús et al. 2009; Navarro-Castilla and Barja 2014a). During the experiment, mean conditions of temperature (10.44 °C), rainfall (1.011 mm), and moonlight (42.36%) were practically constant during the experiment, being the same in the four study plots (AEMet Spanish Weather Service, [www.opendata.aemet.es](http://www.opendata.aemet.es)).

### Live trapping and data collection

Mice were captured during March 2017. Eighty Sherman® live traps were set in four plots separated 35 m. Each plot had 20 traps placed 7 m apart from each other in a 4 × 5 grid. Total trapping effort was 1200 trap-nights (80 traps × 15 nights). All traps were set under vegetation to ensure survival of the mice in case of harsh environmental conditions.

All captured mice were individually weighed with a scale (PESNET, 100 g). Sex and breeding condition were determined according to Gurnell and Flowerdew (2006). Sex was determined by anogenital distance. In adult males, breeding condition was determined by examining whether the testes were descended into the scrotal sac. In adult females, breeding condition was determined based on prominent nipples on the abdomen and thorax, and presence of a perforated vaginal membrane. Harmless waterproof paints (Marking stick DFV, [www.divasa-farmavic.com](http://www.divasa-farmavic.com)) were used to mark individuals in different body regions (e.g., ears, back, toes, and tail) to identify recaptures (Hernández et al. 2018a; Sánchez-González et al. 2018a). All captured animals were immediately released after handling. As our study involved focal animal observations, it was not possible to record data blind.

### Simulation of predation risk by fecal odor

Red fox feces were used to simulate predation risk because this species is one of the most common small mammal predators in the region (Serafini and Lovari 1993; Padial et al. 2002) and because it reliably triggers antipredatory responses in rodents (Dickman and Doncaster 1984; Navarro-Castilla and Barja 2014a, b; Sánchez-González et al. 2017, 2018b). Fresh feces were gathered from captive red foxes (one male and one female) from the Centro de Naturaleza Opennature Cañada Real (Peralejo, Madrid). Only those with a layer of

mucus, an elevated level of hydration, and strong odor were considered fresh (Liu et al. 2006; Martín et al. 2010; Barja et al. 2011). All feces samples were frozen at − 20 °C until treatment preparation. Because volatile compounds vary in relation to seasonal or individual factors (Raymer et al. 1984; Andreolini et al. 1987; Hayes et al. 2006; Scordato et al. 2007), all collected feces were mixed to provide a uniform stimulus across all treated traps.

In each plot, half of the traps were subjected to a predator odor treatment consisting of 2 g of fresh red fox feces. Within the 4 × 5 grids set in each plot, predator treatment was set on two non-consecutive rows (10 traps) while the other two rows (10 traps) acted as controls (i.e., without predator fecal cues). The fecal material was placed on one side of the trap entrance to avoid blocking the entry for rodents but close enough to act as a potential cue of predation risk. Predator treatment was replaced every day at sunset.

### Food access and food intake

For the food access experiments, a control plus four different and consecutive treatments of food restriction were tested. All treatments lasted three consecutive nights and used 5 g of toasted corn as bait. Our main aim was to evaluate animal foraging skills and learning behavior; therefore, instead of randomized treatments along the experiment, we decided to firstly expose individuals to control traps and then, they were subjected to increasing order of food access restriction scenarios. The first three nights, all traps were baited with 5 g of toasted corn freely accessible (control). Later, every three nights, food access was experimentally changed in sequence by providing the 5 g of bait inside: (1) straw balls (8 × 5.5 cm), (2) straw balls wrapped in 1.5 m of metal wire, (3) opened plastic bottles (6 cm length × 2 cm of aperture diameter), and (4) closed plastic bottles with 10 small holes (1 mm) made with a needle to allow mice to smell the bait and obtain it by gnawing the bottles.

The remains of unconsumed bait left by each captured individual were collected and stored frozen. Afterwards, the samples were dried at 80 °C in a heater for 1 h, and then weighed with an electronic balance (C-3000/0.01 g CS, COBOS; precision 0.01 g) to determinate the amount of food eaten in each trap by each mouse.

### Feces collection and quantification of fecal corticosterone metabolites

Fecal cortisol/corticosterone metabolites have been widely used as a reliable non-invasive measure of GC levels (Ylönen et al. 2006; Götz and Stefanski 2007; Escribano-Avila et al. 2013; Woodruff et al. 2013; Navarro-Castilla et al. 2014b; Tarjelo et al. 2015; Navarro-Castilla et al. 2017; Sánchez-González et al. 2018a). To avoid any effect of circadian rhythms in excretion patterns (Touma et al. 2003), fecal sample

collection was always performed at the same time each morning. Peak FCM concentrations can be found in wood mouse feces approximately 10 h after the injection of adrenocorticotrophic hormone (Navarro-Castilla et al. 2018). In a previous study carried out with this species, wood mice were captured in traps on average 6 h after dusk (Navarro-Castilla et al. 2018). Thus, we activated the traps at dusk and daily checked them between 10:00 and 11:30 am to ensure that animals were confined inside traps, enough time to see the possible effect of the stressor reflected in FCM levels. The same study also revealed that individuals captured spent similar times inside traps (Navarro-Castilla et al. 2018), so we have conservatively assumed that the time of confinement was not likely influencing the physiological stress response. We only collected the freshest feces and feces with urine present were discarded to prevent a possible cross contamination that could have affected our results (Touma et al. 2003). During fieldwork, samples were stored in Eppendorf tubes in a portable cooler with wet ice (4 °C). Once we checked all traps, the samples were taken to the laboratory and maintained in storage at - 20 °C.

Following a modification of Navarro-Castilla et al. (2018) methodology for corticosterone extraction, frozen fecal samples were dried in a laboratory oven at 90 °C until they exhibited a constant mass, which took 4 h. After that, all samples were homogenized with mortar and pestle; then, we took 0.05 g of each sample and stored it frozen at - 80 °C until it was assayed. For the assay, steroids were extracted in sterile crystal vials using 1 ml of methanol for each 0.05-g sample on a plate shaker for 17 h (Labnet Orbit P4, 150 rpm). Samples were then centrifuged for 15 min at 1500 rcf (Eppendorf 5810R 15-amp version Centrifuge). Supernatants were diluted 1:100 with assay buffer and the assay was performed immediately after. To analyze the FCM concentration in the extracts, we used a commercial corticosterone enzyme immunoassay (Cayman chemical, Ann Arbor, Michigan). These commercial kits have been validated for rodent serum and feces (Cayman chemical, Corticosterone ELISA kit no. 501320). Nonetheless, samples were measured in duplicate and the assay was validated in the laboratory (parallelism, intra- and inter-assay coefficients of variation). Parallelism was performed with serial dilutions of fecal extracts (1:50, 1:250, 1:1250) resulting in a curve parallel to the standard. The intra-assay precision was 7.6 mean  $\pm$  0.6 SE. For inter-assay precision, we assayed the same two samples (low and high concentrations) on each plate that was used and the coefficient of variation was 20.4%. Results are expressed as picograms of corticosterone metabolites per gram of dry feces matter.

### Statistical analysis

Chi-square ( $\chi^2$ ) tests were used to check the independence between the observed and expected number of captures and recaptures depending on predation risk presence. Differences

in food intake were analyzed using a generalized linear mixed model (GLMM) with normal distribution and identity link. The response variable was food intake (g) by each individual. The categorical predictors considered in the model were predation risk (control/predator treatment), recapture (first capture/recapture), food access (control/straw ball/straw ball wrapped in metal/open bottle/closed bottle), sex (female/male), and reproductive status (breeding/non-breeding). We also considered body mass as a continuous covariate for the model. To assess the HPA response, we used a GLMM with gamma distribution and log link. The concentration of FCM (pg/g dry feces) was set as the dependent variable and the explanatory variables considered for the model were the same as for food consumed: predation risk, recapture, food access, sex, and reproductive status. Body mass of individuals was also considered covariate for the model. Because some individuals were captured several times along the experiment, the individuals nested within experimental treatments (i.e., food access and predation risk) were included as random factors in the GLMMs to control for pseudoreplication and inter-individual variation under the same experimental conditions. Results were considered significant at  $\alpha < 0.05$ . Data are represented as mean  $\pm$  standard error (SE). The software used to perform the statistical analysis was SPSS 23.0 for Windows (SPSS Inc, Chicago, IL, USA).

## Results

### Predation risk avoidance

The total number of captures during live trapping was 146, including 92 recaptures. We found differences in mouse capture and recapture depending on predator cue presence. We had significantly more captures in traps without predator feces (64.81%), whereas only 35.19% of captures occurred under predation risk treatment ( $\chi^2 = 4.741$ ,  $P = 0.029$ ). Recaptures were also more common when predator feces were not present (36.96% vs 63.04%;  $\chi^2 = 6.261$ ,  $P = 0.012$ ).

### Wood mouse food intake

Food access was one of the main factors explaining the food intake (Table 1). Mice showed increased food intake when facing straw balls (3.58  $\pm$  0.27 g;  $N = 24$ ) and open bottles (3.08  $\pm$  0.19 g;  $N = 49$ ), followed by the straw balls with metal wire (2.45  $\pm$  0.30 g;  $N = 23$ ), control (2.10  $\pm$  0.32 g;  $N = 23$ ), and closed bottles (2.04  $\pm$  0.30;  $N = 23$ ) (Fig. 1a). Additionally, we found that recapture also modulated food intake, with recaptured mice consuming more food (2.96  $\pm$  0.15 g;  $N = 89$ ) compared with mice captured the first time (2.36  $\pm$  0.21 g;  $N = 53$ ). However, this result was better explained by the significant interaction between recapture and reproductive status, which showed us that only breeding individuals increased food

**Table 1** Results of the GLMM analyzing the effect of individual, environmental, and experimental factors on wood mice food consumption

Factor	<i>F</i>	<i>df</i>	<i>P</i>
Food access	3.879	4	0.005
Predation risk	1.396	1	0.240
Recapture	4.936	1	0.028
Sex	0.523	1	0.471
Reproductive status	0.005	1	0.945
Mass	1.542	1	0.217
Predation risk × food treatment	0.301	4	0.877
Recapture × food treatment	0.508	4	0.730
Recapture × reproductive status	5.425	1	0.022
Sex × reproductive status	4.498	1	0.036
Individual (nested within experimental treatments) <sup>a</sup>			

<sup>a</sup> Random factor (estimate 0.260, SE 0.297, Z-test 0.877, *P* 0.381)

intake when being recaptured (coefficient ± SE = 1.160 ± 0.498, *P* = 0.022; Fig. 2). Furthermore, the interaction between sex reproductive status resulted significant due to less food consumption of non-breeding females compared with all other groups (coefficient ± SE = 1.050 ± 0.495, *P* = 0.036; Fig. 3). Predator feces did not have any effect on food intake (Table 1).

## HPA response

The analysis of mouse fecal samples revealed that the factors which most contributed to the concentration of fecal corticosterone were food access, sex, and the interaction between sex and reproductive status (Table 2). FCM concentrations were highest in mice exposed to the straw ball wrapped in metal wire treatment (18,955 ± 2585 pg/g; *N* = 25) and lowest in the control treatment (7048 ± 1227 pg/g; *N* = 24). FCM concentrations were similar in the straw ball treatment (13,921 ± 1576 pg/g; *N* = 24), opened bottle treatment (12,192 ± 1396 pg/g; *N* = 49), and closed bottle treatment (11,959 ± 1596 pg/g; *N* = 23) (Fig. 1b). Female mice had heightened FCM levels (14,267 ± 1461 pg/g; *N* = 59) compared with males (11,604 ± 928; *N* = 87) and the significant interaction between sex and reproductive status was due to non-breeding males displaying markedly low FCM concentrations (coefficient ± SE = -0.513 ± 0.242, *P* = 0.036; Fig. 4). On the other hand, predator scent had no effect on FCM levels (Table 2).

## Discussion

### Predation risk avoidance

The results obtained corroborated that wood mice detected and avoided the predator scent since capture likelihood

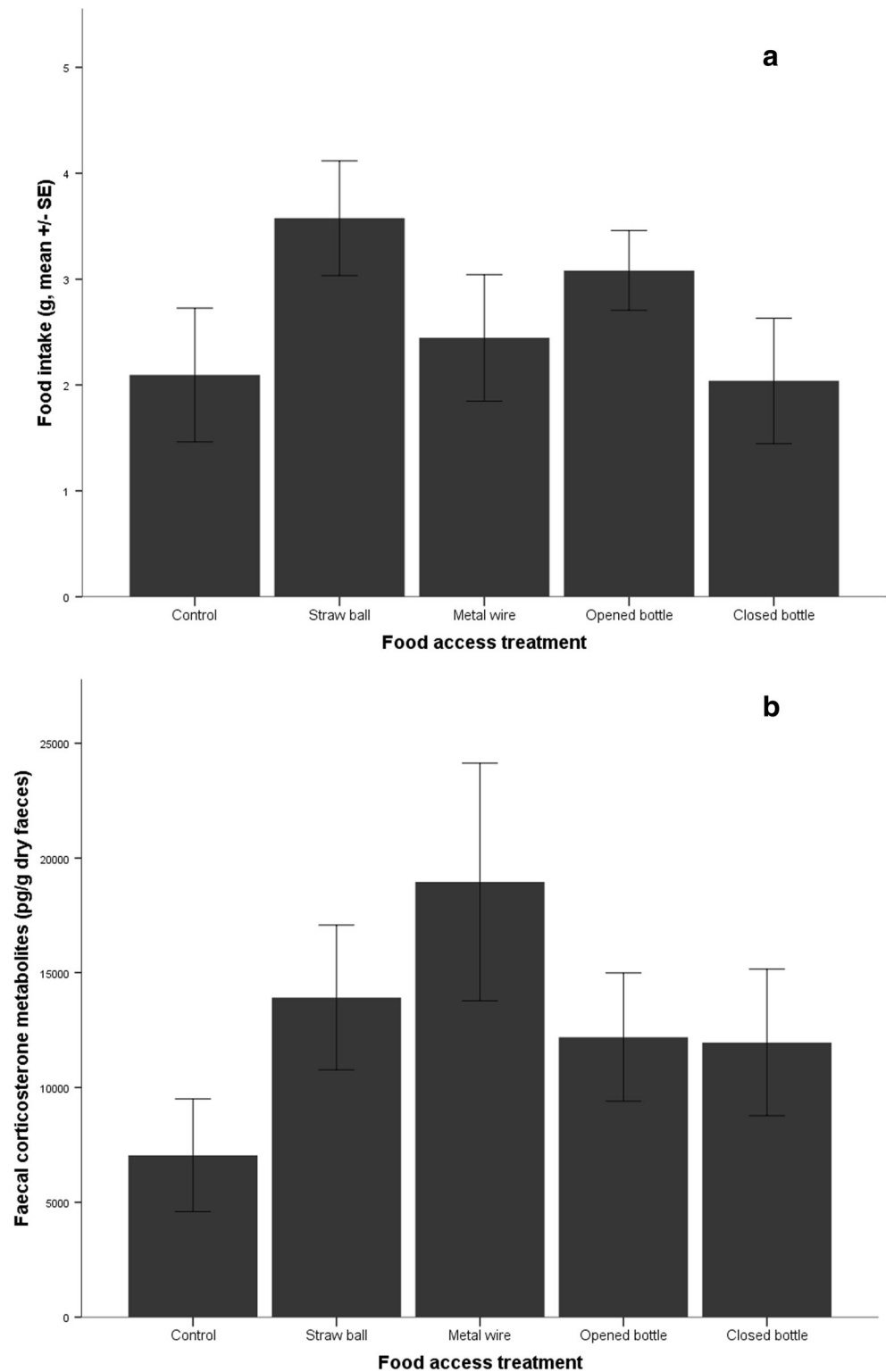
significantly decreased in the presence of red fox feces. The trade-off between foraging and antipredator strategies explains why individuals generally tend to avoid high-risk environments, but this phenomenon varies depending on individual characteristics and needs, and that is why some mice were still captured (Lima and Bednekoff 1999; Hernández et al. 2018b; Navarro-Castilla et al. 2018). Our results are consistent with previous reports in which wood mice avoided direct predator fecal cues (Dickman and Doncaster 1984; Navarro-Castilla and Barja 2014a, b; Navarro-Castilla et al. 2018).

### Wood mouse food intake

Our results suggest that mouse food intake was probably determined by the ease of the access to the bait and the energetic cost needed to obtain it. Individuals ate less in those treatments in which food access was especially difficult (i.e., straw balls wrapped in metal wire and closed plastic bottles). As Anselme et al. (2017) pointed out, stronger handling costs tended to decrease consumption in unpredictable environments; therefore, mice would choose not to spend too much energy when food is almost inaccessible, as gaining partial access to food does not compensate the vast metabolic effort needed to obtain it. On the other hand, Perrigo (1987) showed that mice can adjust their physical efforts to obtain food and meet the energy requirements of a particular context. For example, in treatments in which food access was laborious but more feasible, mice kept trying to access to the food because the energetic cost was not that high and the amount of food obtained would compensate for the efforts made. The intermediate food consumption seen in the controls may be explained by the fact that food access was free; thus, these animals ate less because they did not spend any extra energy trying to acquire it. Further, if they do not spend unnecessary time on feeding, they can allocate that time in other fitness-enhancing activities, e.g., trying to escape, grooming, staying vigilant, or freezing in response to predator odor (Lima and Bednekoff 1999). Nevertheless, we would like to highlight that we did not know the previous energetic state of each mouse, i.e., whether they had feed just before being caught or if by contrast, their responses were driven by an extreme hunger. This may also condition the individual's willingness to approach traps with the predator cue or the persistence displayed in handling each food access treatment. Besides, we need to consider the possible influence of other individual factors such as the fear response and personality traits (e.g., boldness, neophobia, motivation) that might have affected this response.

Despite previous studies showing that predation risk can modify feeding behavior in rodents (Apfelbach et al. 2005; Navarro-Castilla and Barja 2014a; Sánchez-González et al. 2017), simulated predation risk in this study did not have any effect on wood mouse food intake, contrary to our predictions. This result could suggest that once an individual assumes the

**Fig. 1** Food intake (g, mean  $\pm$  SE) (a) and HPA response (pg/g dry feces  $\pm$  SE) (b) under food access experimental manipulation

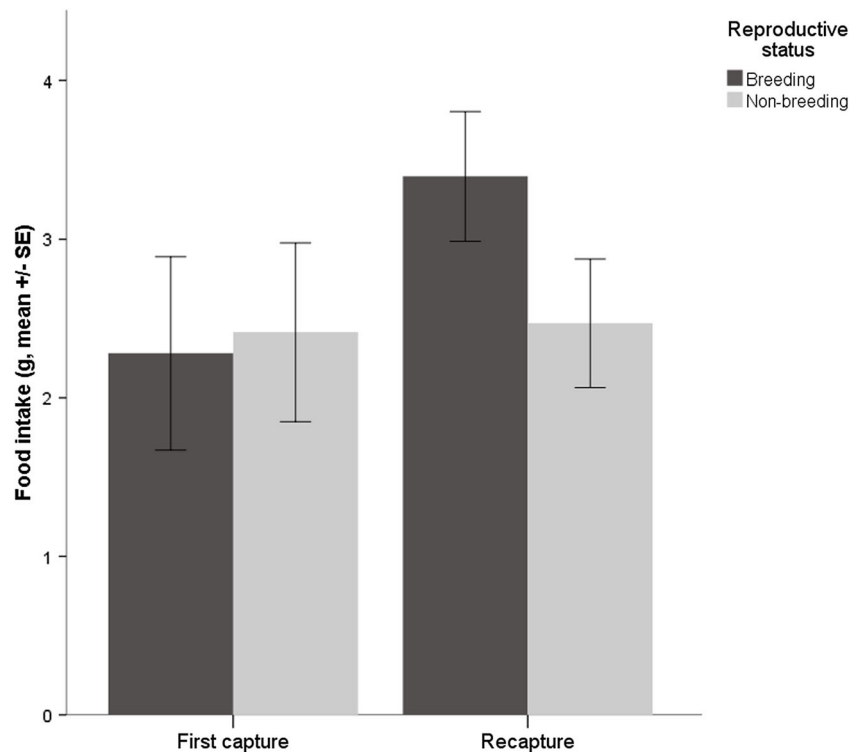


consequences of searching for food during high-risk circumstances, they do not compromise the food intake, consistent with findings in Navarro-Castilla and Barja (2014b) and Navarro-Castilla et al. (2018). Alternatively, the temporal decrease of the predator cues intensity over time, due to

the loss of volatile compounds, may have also likely influence food intake inside traps (Sánchez-González et al. 2017).

We also found that mice captured for the first time ate less than recaptured ones. This result suggests that mice trapped for the first time may be more scared, and they

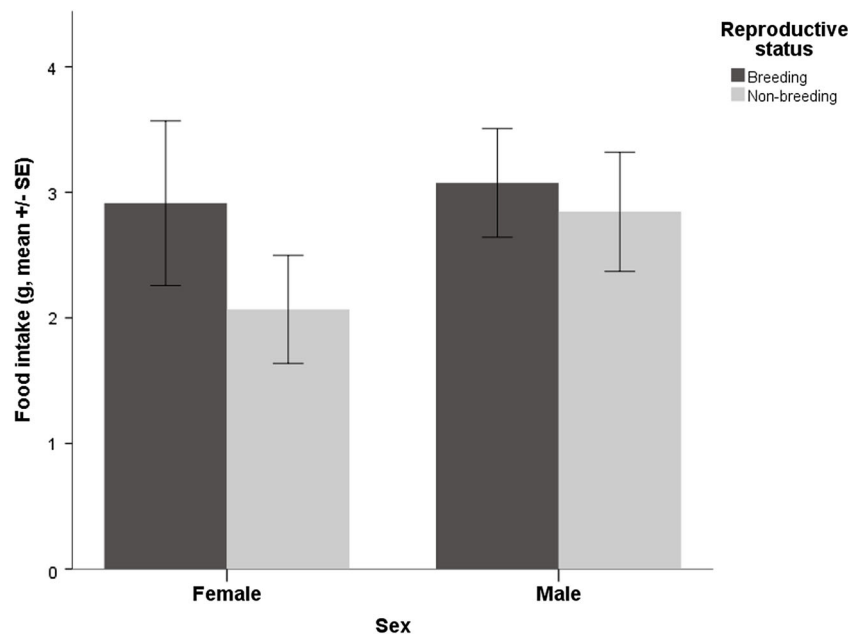
**Fig. 2** Comparison between food intake (g, mean  $\pm$  SE) in recaptured and mice captured the first time depending on the reproductive status of the individuals



are inexperienced with the new situation. Thus, experience plays a key role in food handling by mice and the decisions made to counterbalance the cost of food access. If individuals have previous practice trying to gain food access in a particular setting, they may be able to reach the food more easily and reduce the energy expenditure associated with foraging. Thus, having previous experience entails that mice could eat more even in the most difficult treatments,

since they could compensate the energetic cost associated with a more efficient device handling. However, it seems that only recaptured breeding mice were responsible for this effect, suggesting that when energy requirements are increased due to reproduction (Thompson 1992; Król and Speakman 2003; Liu et al. 2003; Speakman 2007), improving their feeding skills would be exceptionally important to maximize their biological fitness.

**Fig. 3** Wood mice food intake (g, mean  $\pm$  SE) depending on the sex and the reproductive status of the individuals



**Table 2** Results of the GLMM testing the effect of individual, environmental, and experimental factors on fecal corticosterone metabolites in the wood mouse

Factor	<i>F</i>	<i>df</i>	<i>P</i>
Food access	5.047	4	0.001
Predation risk	1.491	1	0.224
Recapture	1.136	1	0.289
Sex	7.669	1	0.006
Reproductive status	0.016	1	0.899
Mass	2.236	1	0.137
Predation risk × food treatment	0.571	4	0.684
Recapture × food treatment	1.151	4	0.336
Recapture × reproductive status	0.107	1	0.745
Sex × reproductive status	4.498	1	0.036
Individual (nested within experimental treatments) <sup>a</sup>			

<sup>a</sup> Random factor (estimate 0.010, SE 0.061, Z-test 0.163, *P* 0.871)

Finally, there was a significant interaction due to less food consumption of non-breeding females compared with all other groups which could be explained by the substantial decrease in the energetic demands outside breeding period, since they would not be gestating or lactating (Künkele 2000; Zenuto et al. 2002). Moreover, males appear to be more aggressive (Hernández et al. 2018a) and agonistic interactions due to a strong competition for suitable territories and for the access to females (Lambin 1988) could lead to higher energetic costs.

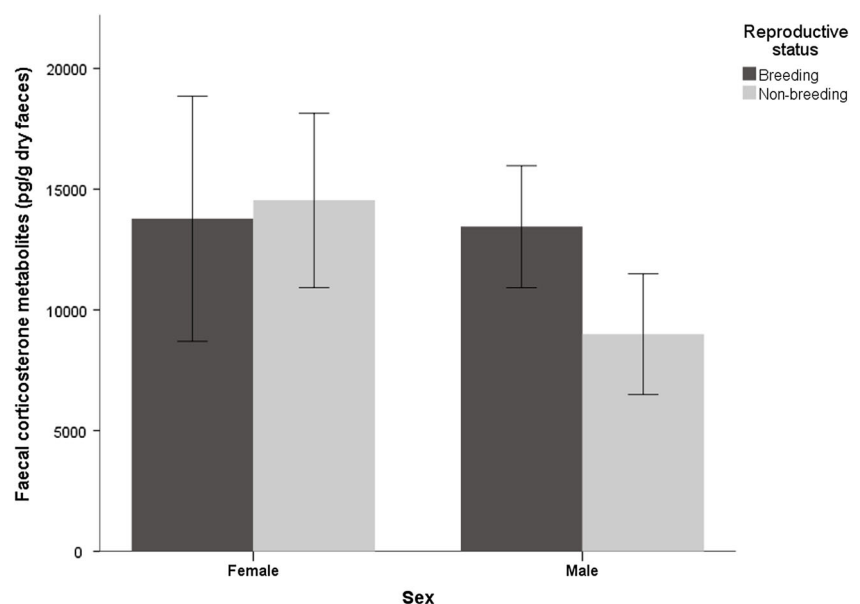
## HPA response

As expected, we found that wood mouse FCM concentrations varied according to the complexity and penetrability of the

food treatments. The lowest FCM levels were found when mice had free access to food (i.e., the control). On the other hand, mice experiencing the straw balls wrapped in metal wire had the highest FCM levels, suggesting that obtaining the food was particularly arduous in this case and mice needed to allocate more energy. Additionally, this treatment could have been especially frustrating for the individuals since the straw balls may have been considered as nesting material and restricted access to it is known to be stressful (Ivy et al. 2008). For the rest of the food access treatments, we observed elevated FCM levels compared with the control. Results suggested that the HPA axis was impacted by the need to increase energy availability in order to gain access to food.

Activation of the HPA axis in prey species exposed to predation risk is well-documented (Perrot-Sinal et al. 1999; Morrow et al. 2000; Harris and Carr 2016). However, the literature on the effects of predator fecal cues on GC levels in rodents is not conclusive. On one hand, there is clear demonstration that predator olfactory signals trigger the physiological stress response (Monclús et al. 2005; Sánchez-González et al. 2018b), while others were unable to detect this pattern (Fletcher and Boonstra 2006; Ylönen et al. 2006; Navarro-Castilla 2014b; Navarro-Castilla et al. 2018). Contrary to our initial hypothesis, we did not observe any influence of simulated predation risk on the wood mouse FCM levels in this study. A possible explanation is that predator scent cues are not always sufficiently strong or reliable without further information (as per Orrock et al. 2004; Fletcher and Boonstra 2006). Moreover, as Navarro-Castilla et al. (2014b) indicated, in natural environments, prey often come across predator odorous signals, and repeatedly triggering the physiological stress response could lead to chronic stress, decreasing individual fitness. In our

**Fig. 4** Mean fecal glucocorticoid metabolites (pg/g dry feces) ± SE in relation to the sex and the reproductive status of the individuals





experiments, activating the HPA axis may result in important costs that could compromise feeding. Hence, in this specific situation where mice are sheltered inside the traps (Sánchez-González et al. 2017; Hernández et al. 2018b), the benefits of focusing on feeding could be greater than evoking an antipredator response via elevated GC.

As we expected, FCM levels also varied depending on individual factors such as sex. Females exhibited increased FCM concentrations, a pattern which is consistent with previous studies with different rodent species (Navarro-Castilla et al. 2014a, b, 2017) and which can be explained by differences in the metabolism of glucocorticoids between sexes (Touma et al. 2003). However, these differences also appear to be driven by the significant interaction between sex and reproductive status, which revealed that non-breeding males exhibited diminished FCM concentration. We hypothesize that energetic requirements and GC concentrations of non-breeding males were lower because they do not have to engage in mating competition nor intraspecific agonistic interactions (Scott 1987; Haller et al. 1998; Fano et al. 2001).

To the best of our knowledge, this is the first report showing evidence of the ability of mice to balance their energy budget depending on food access difficulty. When obtaining food is arduous, individuals compensate for the energy expenditure by increasing the amount of food eaten. However, if the cost of gaining food access is exceedingly high or low, they eat less. In the former case, this is likely because the energy acquired does not counterbalance the costs. In the latter case, because they do not have to expend much energy to obtain food. Furthermore, our results evinced that experience is a crucial feature in mice regarding their foraging and energy budget optimization. Also, the variation in FCM levels according to food access difficulty implies that GC release can be an effective mechanism to cope with feeding challenges. We would like to highlight that although we carried pilot trials to evaluate food access treatments' difficulty (by means of food intake and treatment aspect after mice manipulation), we could not perform direct observations inside traps, i.e., evaluating mice behavior and food/treatment management. Therefore, our human perception of the degree of restriction between treatments may vary with respect to what mice actually did experience, likely conditioning partially our results. Finally, though mice avoided fox-scented traps, direct predation risk cues did not affect food intake or the HPA response, perhaps because in this context, feeding would be more fitness-rewarding than triggering antipredatory strategies. Conducting experiments in the wild entails some limitations. Thus, we acknowledge that for example the lack of complete control of individual variability and the effect of other external factors might have partially influenced both behavioral and physiological responses.

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**Data availability** The dataset analyzed during the current study are available from the corresponding author on reasonable request.

## Compliance with ethical standards

**Ethical approval** This research complies with the regulations on the protection of animals used for scientific purposes (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 and the Spanish legislation (Royal Decree 53/2013)). The study had the approval of the Autonomous Community of Madrid (reference number 10/240775.9.16) and favorable reports from the Ethics Committee of the Autonomous University of Madrid (CEI 73-1330).

**Conflict of Interest** The authors declare that they have no conflict of interest.

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