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Behavioural and physiological responses of wood mice (*Apodemus sylvaticus*) to experimental manipulations of predation and starvation risk

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HIGHLIGHTS

- Mice exposed to predation risk reduce their body weight, via decreasing food intake.
- Starvation risk induced behavioural changes, but not post starvation hyperphagia.
- Mice provide limited support for the starvation-predation trade-off model.

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ABSTRACT

Body weight and the levels of stored body fat have fitness consequences. Greater levels of fat may provide protection against catastrophic failures in the food supply, but they may also increase the risk of predation. Animals may therefore regulate their fatness according to their perceived risks of predation and starvation: the starvation–predation trade-off model. We tested the predictions of this model in wood mice (*Apodemus sylvaticus*) by experimentally manipulating predation risk and starvation risk. We predicted that under increased predation risk individuals would lose weight and under increased starvation risk they would gain it. We simulated increased predation risk by playing the calls made by predatory birds (owls: *Tyto alba* and *Bubo bubo*) to the mice. Control groups included exposure to calls of a non-predatory bird (blackbird: *Turdus merula*) or silence. Mice exposed to owl calls at night lost weight relative to the silence group, mediated via reduced food intake, but exposure to owl calls in the day had no significant effect. Exposure to blackbird calls at night also resulted in weight loss, but blackbird calls in the day had no effect. Mice seemed to have a generalised response to bird calls at night irrespective of their actual source. This could be because in the wild any bird calling at night will be a predation risk, and any bird calling in the day would not be, because at that time the mice would normally be resting, and hence not exposed to avian predators. Consequently, mice have not evolved to distinguish different types of call but only to respond to the time of day that they occur. Mice exposed to stochastic 24 h starvation events altered their behaviour (reduced activity) during the refeeding days that followed the deprivation periods to regain the lost mass. However, they only marginally elevated their food intake and consequently had reduced body weight/fat storage compared to that of the control unstarved group. This response may have been constrained by physiological factors (alimentary tract absorption capacity) or behavioural factors (perceived risk of predation). Overall the responses of the mice appeared to provide limited support for the starvation–predation trade-off model, and suggest that wood mice are much more sensitive to predation risk than they are to starvation risk.

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1. Introduction

Obesity has become one of the major public health problems in western societies [1]. This has led to a need for a better understanding of the mechanisms that underpin the regulation of body weight and fatness [2,3]. A balance between energy intake and energy expenditure is necessary to maintain a stable body weight. It is well established that the central nervous system (CNS) regulates food intake and energy expenditure in response to neuronal, hormonal and nutrient signals [4,5]. However, the exact mechanisms by which these signals are integrated and hence regulate the overall levels of adiposity remain elusive.

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There have been several theoretical models that have attempted to describe the underlying mechanisms involved in the regulation of body fatness. Among the earliest of these was the 'lipostatic set point' model. This model suggests that the size of body fat depots is sensed by a 'lipostat', which adjusts food intake and energy metabolism to maintain the body and fat masses at a set-point [6]. This model requires a signal from the body that indicates to the brain the levels of body fat and a 'set point' in the brain that allows an appropriate response to these peripheral fat level — increasing them if they are too low, by a stimulation of intake and inhibition of expenditure, and decreasing them if they are too high by the reverse processes. Although the hormone leptin, produced primarily in white adipose tissue [7,8] has been often suggested to be the peripheral fat signal in this model, a central location for the 'set point' has never been identified. Moreover, this model is in conflict with observations of the patterns of changes in animal and human body weight [9,10] not least of which is the obesity epidemic itself [2].

An alternative interpretation suggests that body weight and fat mass are not regulated by a set-point, but rather are free to vary dependent on environmental factors, but are constrained to fall between upper and lower intervention points, above and below which animals (and humans) intervene physiologically to bring their body weight and fatness back into the acceptable range [11–13]. In humans the upper intervention point may be located at different positions in different individuals, explaining why some individuals become obese when exposed to environments with readily available food supplies, but others are able to regulate their body weights at normal levels [14]. Based on a wealth of data from small mammals and birds e.g. [15,16], it has been suggested that the lower intervention point may be defined by the risk of starvation, while the upper intervention point may be defined by the risk of predation [12,14].

The starvation–predation trade-off model predicts that an increased risk of predation would decrease the upper intervention point, and animals would tend to regulate their weight at lower levels. Conversely, an increase in starvation risk would increase the lower intervention point, and animals would regulate their body weights and fat mass at higher levels. We aimed in the present study to experimentally manipulate both predation risk and starvation risk to test these predictions.

2. Material and methods

Animal capture was authorized by the Institute of Nature Conservation and Biodiversity, Lisbon, Portugal (licence ICNB 231/2010/CAPT); experimental procedures were conducted in the University of Lisbon facilities by an expert in laboratory animal science accredited by the Portuguese Veterinary Authority (1005/92, DGV-Portugal, following FELASA category C recommendations), according to the European guidelines (86/609/EEC).

2.1. Predation risk

Adult wood mice *Apodemus sylvaticus* used in the predation experiment were captured near Portalegre — Portugal (N 39°17′ W 7°26′), using Sherman traps, and brought to the lab in the University of Lisbon where they were housed in individual cages, under controlled light (12D:12L) and temperature (20 °C); a small plastic box was provided for nesting and shelter. Animals were fed ad libitum, with commercial pellets for laboratory mice (Maintenance diet — Scientific Animal Food Engineering, France), and had free access to drinking water. After a three week acclimation period, animals were randomly assigned into experimental groups. A total of 29 animals, 13 males and 16 females, were used in the tests. Due to the limited number of individuals, each animal was included in 2–3 of the five experimental groups and the order of the treatments was randomly selected, as follows: control (silence), blackbird sound during the night, owl sound during the night, owl sound during the day and blackbird sound during the day. After each testing period animals were allowed a recovery period of two weeks, to avoid interference of previous treatment over the trials. During the recovery time, housing and maintenance conditions were as described above and mice were not handled, except for the weekly bedding change. Exposure to owl calls during the daytime was tested in 22 animals; exposure to owl calls at night was tested in 18 animals; exposure to blackbird calls during the daytime was tested on 18 individuals; and exposure to these calls in the day was tested on 10 animals. Finally 10 animals were measured without exposure to any bird calls.

2.1.1. Predation risk treatment

Elevated predation risk was simulated by exposing the animals to a playback call lasting 2 min from nocturnal predators observed at the location in the field where the mice had been captured. The 2 minute call period started at 11 pm and was repeated every 2 h until 7 am the next day. Playback sounds from barn owl *Tyto alba* and eagle-owl *Bubo bubo* were obtained from commercial digital recordings of bird songs (European bird calls; Jean C. Roché, Kosmos Verlag, Stuttgart, Germany). Speakers were placed 3 m away from the mice, and the sound level was adjusted by human ear, to guarantee that sound was heard by the animals. A second group was exposed to a 2 minute long playback of calls from the blackbird *Turdus merula*, a non-threatening bird species, also observed at the mouse capture site. The exposure procedure and setup were the same as described above for the predation risk group.

The third and fourth treatment groups were exposed to the broadcasting procedure described above, using owl and blackbird calls, but the exposures were made during daytime. The daytime playback calls were broadcasted during a 2 minute period, every 2 h, starting at 11 am until 7 pm. Animals in a further control group were kept in silence during the entire period of the experiments. The period of exposure to the treatment lasted 5 days.

2.1.2. Body weight

Mice were weighed daily between 3 pm and 6 pm each day. Since mice were exposed to repeated treatments they could not be dissected at the end of each experimental period to obtain direct measurements of body fatness.

2.1.3. Food intake and dry mass absorption efficiency

Food intake and digestibility were quantified in mice from the five experimental groups. At the beginning of the test, each animal was weighed and placed in a clean individual cage (30 cm × 20 cm). The cage floor was covered with absorbent paper, and food was placed in excess in the food hopper. After 24 h, the food left in the hopper was weighed, as well as any food remains on the cage floor. Faeces were collected, weighed and dried at 70 °C, until the weight was stabilized (≈72 h). The procedure was repeated for the 5 consecutive days of the experiment, between 3 pm and 6 pm each day. Apparent dry mass absorption efficiency was calculated as the difference between food intake and faecal output divided by food intake e.g. [17,18].

2.1.4. Faecal corticosterone levels

On the sixth day, after the trials had finished, animals were placed in a clean cage and fresh faeces were collected for measurement of corticosterone levels. Faeces were stored in absolute ethanol at −30 °C, until being processed. The faecal concentration of corticosterone peaks about 6–12 h after a stressful event [19], thus faeces were collected between 10 am and 1 pm for animals in the control group and exposed to the sound treatment during nighttime, and between 5 pm and 8 pm for animals exposed to the sound treatments during daytime.

Hormone extraction was performed following a modified method of Goymann [20]. Briefly, ~0.3 g of faeces (Sartorius) was added to 4 ml of methanol, and pulverized using a small pallet knife. The mixture was then vortexed for 1 h at 500 rpm, followed by 1 h of centrifugation at 6000 rpm. The supernatant was then transferred to another tube and

diluted with the buffer solution from an EIA Kit. Corticosterone levels were determined using Enzyme Immunoassay (EIA) commercial kits (ADI-900-097, Assay Designs).

2.1.5. Resting metabolic rate

Oxygen consumption was measured, after 6 days of experimental treatment, using an open-flow respirometry system (Servomex, series 4100) as previously described [21]. Briefly mice were placed in a cylindrical chamber, and dried atmospheric air was pumped into the chamber at a flow rate of 500 ml/min. Ambient temperature was set at 29 °C, in the thermoneutral zone [22]. Each animal was continuously monitored, during 2 h for two consecutive days, during the daytime when wood mice are normally inactive [23]. No food or water was available inside the chamber. Measurements of oxygen concentration were digitised approximately 35× per second and the accumulated data averaged over 30 s intervals (mean of approximately 1000 measurements). These 30 s averages were then saved. Resting metabolic rate was estimated as the average value of the five lowest consecutive readings (equivalent to 2 min and 30 s in the chamber) [24], and the average of the measurements made on consecutive days was used for further analysis. VO_2 was calculated after Depocas and Hart [25] as $VO_2 = V_2(F_1O_2 - F_2O_2) / (1 - F_1O_2)$, where V_2 is the flow rate measured after the metabolic chamber, and F_1O_2 and F_2O_2 are the oxygen concentrations before and after the metabolic chamber. All the values were corrected to standard temperature and pressure (STPD). Baseline values of atmospheric oxygen were corrected by a 30 minute measurement prior to each run.

2.2. Starvation risk

2.2.1. Animals and experimental design

The 30 mice used in this study were the first generation descendants of the mice used in the experiment on predation risk. After weaning at 21 days of age, animals were separated from their mothers, and housed in individual cages, with wood shavings for bedding, a small plastic box and shredded paper for enrichment. Ambient temperature was controlled at 20 °C and the light cycle was 12L:12D. Food and water were provided ad libitum, and the animals were fed on commercial chow pellets (Scientific Animal Food Engineering, France).

When the animals reached 12–14 weeks old, they were allocated into one of two groups: the control group (6 males and 6 females) and the stochastic starvation group (9 males and 9 females). Animals in the control group were fed daily with 50 g of chow pellets. Every day uneaten food on the feeder and cage floor was collected and weighed and the initial amount was replaced into the feeder. The procedure was repeated for a total of 18 days. Body weight was also monitored on a daily basis. At days 7 and 14 the faeces were collected, dried for 48 h at 70 °C and weighed. Animals in the starvation group were fed following the procedure described above, the first 3 days were established as baseline, and then they were submitted to a set of stochastic food deprivation days. The probability of having a starvation day was preestablished as 0.28 corresponding to a total of 4 days within 15 remaining days of the study. Each starvation day was followed by a feeding day; starvation days corresponded to days 6, 9, 13 and 15. In the starvation days all the food was removed from the feeder and replaced 24 h later. For the starvation group, faeces were collected at days 4, 7 and 14. After collection, faeces were dried for 24 h at 80 °C, and dry mass was measured using a 4 figure balance (Sartorius). Dry mass absorption efficiency was calculated as the difference between food intake and faecal output divided by food intake e.g. [17,18].

2.2.2. Plasma assays

After the 18 days, all the animals were starved for 4 h before the collection of approximately 1 ml of blood by cardiac puncture, followed by sacrifice through cervical dislocation. Samples were collected between 4 pm and 6 pm, and blood was immediately centrifuged for 10 min at 20,000 rpm. Plasma was then stored at −80 °C until processing. Plasma leptin levels were measured using the ELISA based method, with commercially available kits (Millipore Corporation, USA —mouse leptin kit). After blood collection, animals were dissected, and full body fat was collected and weighed.

2.2.3. Behavioural observations

Animals were video recorded over an 8 hour period on multiple occasions (4 h of light and 4 h of dark). The 8 hour period coincided with two different times of day. In the afternoon cameras were set 2 h before the dark phase, and allowed to operate for 4 h, while in the morning cameras were set 2 h before the light phase, and allowed to operate for 4 h. As mouse vision is limited at wave lengths of light below 630 nm [26], dark phase records were made under red light illumination (Phillips, Infrared PAR38).

The control group was observed three times, on days 2, 4 and 9. The starvation group was observed 5 times, including free feeding days, starvation days, and refeeding days. Within each of the recorded films, each animal was observed for 5 random periods of 10 min, and the time spent on each of categorized behaviours was registered. Dominant behaviours were classified into four categories: grooming, feeding, resting and general activity, following the ethogram previously described for mice (see [27,28] for details). General activity included walking, climbing the cage and all general movements. Feeding included eating chow, drinking and occasional coprophagia. Resting was considered when the animal was sleeping or sitting, was not moving in the cage and was not grooming. Grooming behaviour was registered when the animals were not moving and included licking the fur and tail and scratching with any limb.

2.3. Statistical analyses

2.3.1. Predation risk

All the data are expressed as mean ± S.E. Mixed modelling followed by pairwise comparisons was used to compare body weight, food intake, and apparent absorption efficiency variation across the 5 experimental days; treatment and day of measurement were included in the model as fixed factors. Body weight was included as a covariate, for the analysis of food intake variation. Individual ID was included in the models as a random factor, to account for repeated measures. The influence of treatment on RMR and cumulative food intake was analysed through mixed modelling, setting body weight as a covariate, and individual ID as a random factor due to the repeated measurement of individuals across treatments. One-way ANOVA was used to compare corticosterone levels on the last day of treatment, using group as a fixed factor. Due to the wide range of body weight across individual animals (14–62 g), body weight variation was analysed as change relative to day zero of the study. Given the unbalanced number of males and females across the treatment groups, and the reduction of statistical power, sex was not included in the models as an independent predictor.

2.3.2. Starvation risk

All the data were expressed as mean ± S.E. Linear mixed modelling was used to test the body weight and food intake differences between groups and sexes, over the 18 days of the study, and individual ID was included as a random factor to account for the repeated measures. On the analysis of food intake, body mass was included as a covariate in the model. One-way analysis of variance (ANOVA) with post hoc Tukey tests was used to test group differences in body weight and food intake, between time points when group differences were significant over the entire time course.

Differences in cumulative food intake and dry mass absorption efficiency were tested using one-way analysis of variance (ANOVA) with post hoc Tukey tests. Analysis of covariance (ANCOVA) was performed to test differences in plasma leptin levels and body fat between treatment groups, and body fat mass and body weight were used as

covariates accordingly. Dominant behaviours were registered with the Etholog 2.2.5 software [29] and analysed using a GLM model followed by Tukey post hoc tests.

All data were analysed using SPSS 19.0 for Windows.

3. Results

3.1. Predation risk

Animals exposed to the sounds during the night, both owl and blackbird, significantly reduced their body mass, when compared with the control 'silence' group ($p < 0.001$).

Owl calls during the night caused an average of 8% (S.E. = 1.2; $p < 0.001$) reduction in the body weight, and the blackbird calls a 6% (S.E. = 1.6; $p < 0.001$) reduction (Fig. 1). Body weight variation between silent controls, and animals exposed to either owl or blackbird calls during the day, was not significantly different ($p = 0.659$; $p = 0.293$). The variation in the body weight was mostly explained by time (day of experiment effect: $F_{5,32} = 8.188$; $p < 0.001$), treatment ($F_{4,32} = 30.031$; $p < 0.001$), and the interaction between day of measurement and treatment ($F_{20,32} = 2.386$; $p = 0.001$).

The average food intake on the first day of the study was 3.18 g across all the groups. Variations in food intake were mostly explained by day of measurement ($F_{4,28} = 4.322$; $p = 0.002$), treatment ($F_{4,28} = 14.979$; $p < 0.001$), body mass ($F_{1,28} = 10.077$; $p = 0.002$) and the interaction between day of measurement and treatment ($F_{1,28} = 2.157$; $p = 0.006$). Animals in the control group (3.38 ± 0.153 g) and animals exposed to the treatment during the day (predator: 3.49 ± 0.129 g; non-predator: 3.730 ± 0.133 g) had higher food intake than mice submitted to the treatment during nighttime (predator: 3.21 ± 0.131 g; non-predator: 2.82 ± 0.148 g) (Fig. 2).

Differences in cumulative food intake after the 5 days of treatment were explained by different treatments between groups ($F_{4,8} = 5.435$; $p = 0.001$), and the effect of body mass ($F_{1,66} = 14.059$; $p < 0.001$). Cumulative food intake was lower in mice submitted to the treatment during the night (predator: 15.6 ± 0.79 g; non-predator: 13.6 ± 1.0 g) and greater in animals in the control group (16.7 ± 1.0 g) and exposed to the treatment during the day (predator: 17.6 ± 0.8 g; non-predator: 18.4 ± 0.8 g) (adjusted for BM = 28.4 g).

Apparent dry mass absorption efficiency (control = 81.7 ± 0.39; predator day = 80.5 ± 0.24; predator night = 80.4 ± 0.63; non-predator day = 81.3 ± 0.32; non-predator night = 81.7 ± 0.61) showed no significant differences over the 5 days of the study ($F_{4,27} = 1.171$, $p = 0.323$), and no influence due to the treatment ($F_{4,27} = 0.935$, $p = 0.444$). Resting metabolic rate was predominantly influenced by body weight ($F_{1,8} = 47{,}681$, $p < 0.001$), and was not affected by the experimental treatment ($F_{4,8} = 1.021$, $p = 0.403$) (Fig. 3).

Corticosterone levels varied significantly with the treatment group (ANOVA: $F_{5,49} = 7.692$; $p < 0.001$). Levels were highest in the groups exposed to the playback during daytime, of both owl (257.9 ± 53.90 ng/ml) and blackbird (155.0 ± 22.3 ng/ml) sounds (Tukey $p = 0.308$). In contrast, exposure to sounds at night (owl: 47.4 ± 12.85 ng/ml; blackbird: 34.0 ± 14.2 ng/ml) did not result in elevated corticosterone levels relative to the silent control group (41.0 ± 14.1 ng/ml) (Tukey $p = 0.166$).

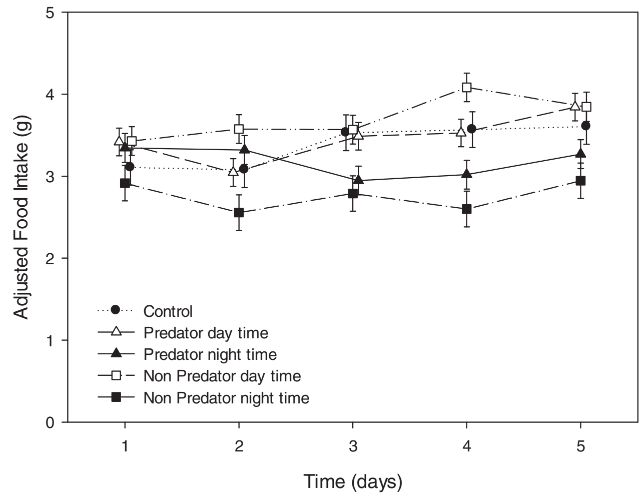

Fig. 2. Variation of food intake (g) (adjusted for BM = 28.29 g; mean ± S.E.), in response to exposure to predator and non-predator sounds played during the day or at night across the 5 days of the study. The control group was exposed to silence.

3.2. Starvation risk

3.2.1. Body weight

The body weight variation was mostly explained by day of measurement ($F_{18,78} = 2.659$, $p < 0.001$) and the interaction between treatment and day of measurement ($F_{18,78} = 9.204$ $p < 0.001$), and the effect of sex is not significant ($F_{1,78} = 0.71$, $p = 397$).

During the first six days of the experiment, prior to the starvation periods, animals in the two groups did not differ significantly in body

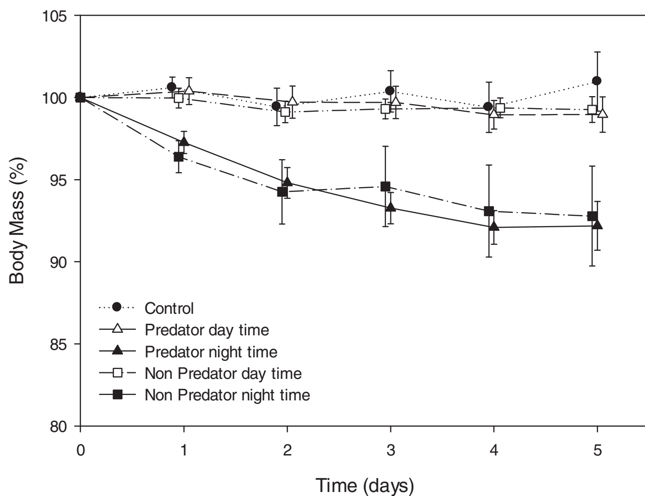

Fig. 1. Changes in body mass (mean ± S.E.) relative to baseline in response to exposure to predator and non-predator sounds played during the day or at night across the 5 days of the study. The control group was exposed to silence.

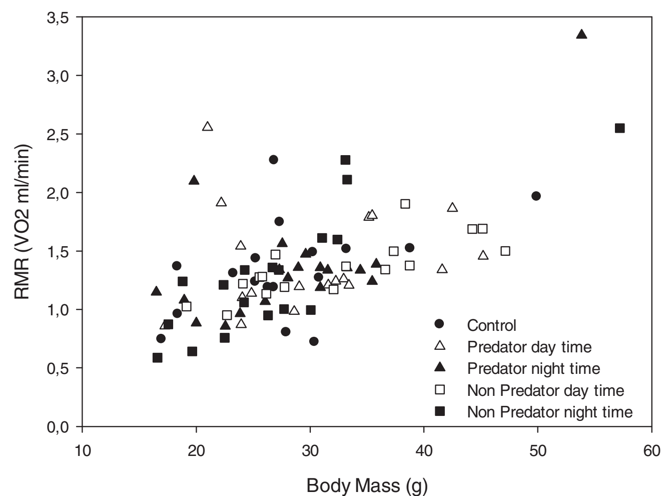

Fig. 3. Effects of body mass (g) on resting metabolic rate (ml O_2/min) for all the measured individuals. Experimental treatment had no significant effect on the resting metabolic rate ($F_{4,8} = 1.021$, $p = 0.403$).

Table 1
Mean ± S.E. of body mass (g) and ANOVA results, on starvation (day 6, 9, 13 and 15) and refeeding (day 7, 10, 14 and 16) days of the study.

		Control	Starvation	ANOVA
Starvation days	Day 6	28.52 ± 1.87 g	26.82 ± 1.62 g	$F_{1,26} = 42.905$; $p < 0.001$
	Day 9	27.85 ± 1.81 g	26.21 ± 1.55 g	$F_{1,26} = 6.482$; $p = 0.017$
	Day 13	28.25 ± 1.74 g	25.02 ± 1.50 g	$F_{1,26} = 110.353$; $p < 0.001$
	Day 15	27.81 ± 1.67 g	24.20 ± 1.45 g	$F_{1,26} = 78.354$; $p < 0.001$
Refeeding days	Day 7	28.44 ± 1.38 g	27.27 ± 1.69	$F_{1,26} = 18.453$; $p < 0.001$
	Day 10	27.77 ± 1.39 g	26.57 ± 1.67	$F_{1,26} = 2.833$; $p = 0.104$
	Day 14	27.97 ± 1.39 g	26.91 ± 1.53	$F_{1,26} = 57.716$; $p < 0.001$
	Day 16	27.78 ± 1.42 g	27.08 ± 1.63	$F_{1,26} = 126.303$; $p < 0.001$

weight (control, 29.1 ± 0.55 g; starvation, 29.5 ± 0.45 g; ANOVA $F_{1,26} = 0.198$, $p = 0.657$). Animals in the starving group significantly decreased their body weight on the deprivation days (Table 1).

The first and second starvation days resulted in a reduction of 8% in the body weight; on the third and fourth starvation days the animals lost 12% of their body weight (Fig. 4).

Except for the day following the second 24 h starvation period (day 10: GLM: $F_{1,26} = 2.8$, $p = 0.104$), the body weight on the first refeeding days was also significantly reduced relative to the control animals. On average the body weight took 2–3 days to recover to control levels after the 24 h starvation period. At the end of the experimental period, body fat of the control group (1.7 ± 0.23 g) was higher than in the starved animals (1.58 ± 0.198 g). These differences were mostly explained by total body weight (ANCOVA: $F_{1,25} = 23.3$, $p < 0.001$), and also by an effect of the treatment ($F_{2,25} = 3.4$, $p = 0.048$).

3.2.2. Food intake

The analysis of food intake revealed that its variation is mostly explained but the interaction of treatment across days of the study ($F_{17,75} = 70.214$, $p < 0.001$), effects due to sex ($F_{1,75} = 0.798$, $p = 0.377$) and body mass ($F_{1,75} = 3.602$, $p = 0.061$) did not significantly affect the food intake.

During the 6 initial days, when all animals were fed ad libitum, the two groups did not differ in the average quantity of food consumed (control, 3.9 ± 0.15 g/day; starvation, 4.0 ± 0.12 g/day; ANOVA: $F_{1,147} = 0.038$, $p = 0.846$) (Fig. 5).

Over the first day of refeeding (day 6), after the first starving period, there were no significant differences in the food intake between the control group and starvation group (control, 4.3 ± 0.28 g; starvation, 4.4 ± 0.23 g; ANOVA: $F_{1,28} = 0.063$, $p = 0.804$). Food intake on the first day of refeeding (day 10), after the second deprivation day (day), was also not significantly different between the two groups (control, 4.1 ± 0.29 g; starvation, 4.9 ± 0.24 g; ANOVA: $F_{1,28} = 3.921$, $p = 0.058$). However, on the first refeeding day, after the third (day 14) and fourth (day 16) days with no access to food, food intake in the starvation group was elevated compared with the control group (day 14: control, 4.5 ± 0.24 g; starvation, 5.4 ± 0.20 g; ANOVA: $F_{1,27} = 8.476$, $p = 0.007$; day 16: control, 4.1 ± 0.23 g; starvation, 5.8 ± 0.197 g; ANOVA: $F_{1,26} = 30.848$, $p < 0.001$). At the end of the baseline period of 5 days, cumulative food intake (Fig. 6) was not significantly different between the two groups (control, 19.4 ± 1.51 g; starvation, 19.9 ± 1.23 g; ANOVA: $F_{1,28} = 0.043$, $p = 0.836$). Cumulative food intake on day 12 (after 2 starvation events), was also not significantly different between the two groups (control, 49.5 ± 2.71 g; starvation, 43.1 ± 2.213 g; ANOVA: $F_{1,28} = 2.214$, $p = 0.079$), but showed a trend ($p > 0.05 < .1$) towards being greater in the control group. On the last day of the study, cumulative food intake of the control group was significantly higher than the starvation group (control, 77.0 ± 3.67 g; starvation, 62.8 ± 3.00 g; ANOVA: $F_{1,28} = 8.94$, $p = 0.006$). The shortfall in intake of the starved group relative to the controls over the 18 day study was 14.2 g of food, almost equal to the daily intake during baseline (3.94 g/day) multiplied by the number of starvation days (=4) (3.94 × 4 = 15.8 g). Therefore overall the intermittently starved mice did not compensate their food intake during the days that they had food available for the days when food was absent (Fig. 6).

Apparent dry mass absorption efficiency was not significantly different between the groups (control = 0.804 ± 0.004; refeeding = 0.815 ± 0.005: $F_{1,71} = 0.016$; $p = 0.899$). Moreover within the starvation group, the starvation/refeeding treatment did not significantly affect apparent dry mass absorption efficiency ($F_{1,71} = 1.680$; $p = 0.199$).

3.2.3. Behaviour and activity

General activity was significantly reduced on the starvation day and the first refeeding day in the starved animals, compared with the

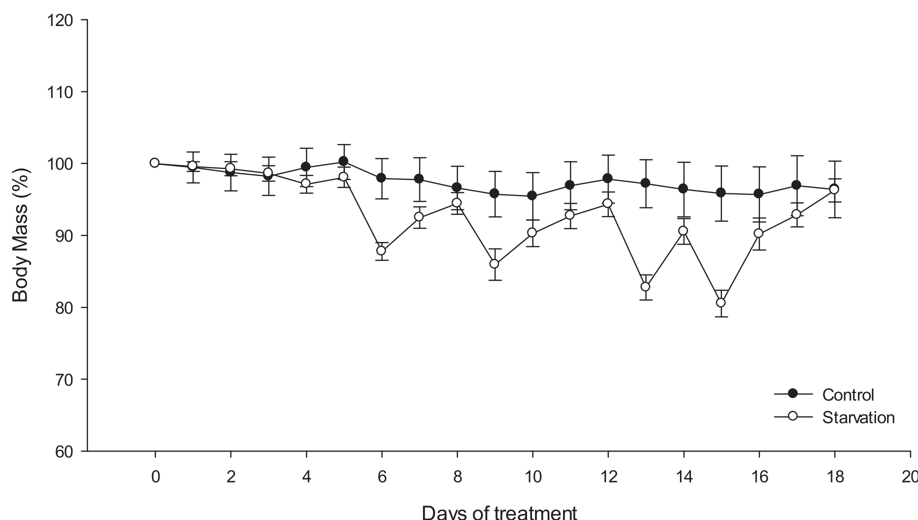

Fig. 4. Effects of stochastic exposure to starvation on the body mass variation of the wood mice, across the 18 days of the study.

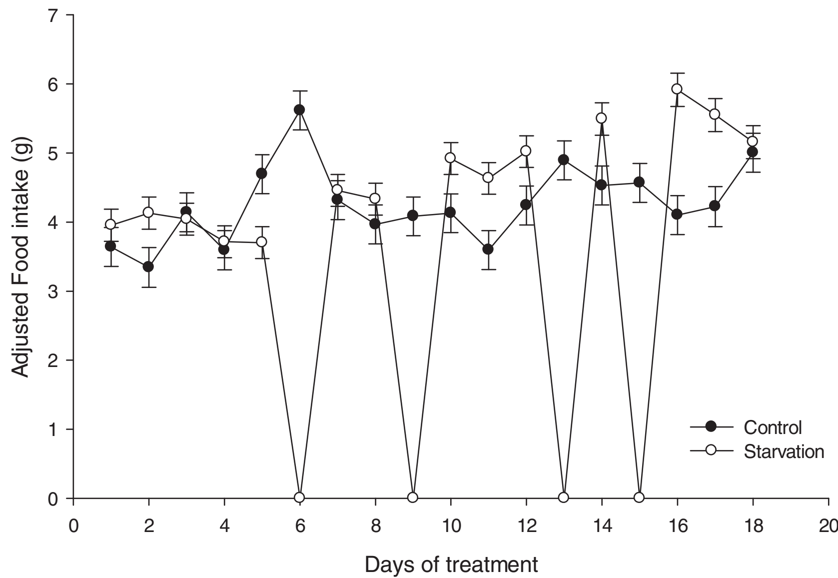

Fig. 5. Effects of stochastic exposure to starvation on the food intake (g) variation over the 18 days of the study (adjusted for BM = 27.85 g).

control data ($p = 0.001$ and $p = 0.004$ respectively). The difference in general activity levels between the starvation day and the first refeeding day was not significant ($p = 0.202$). Time spent resting was significantly increased on starvation ($p = 0.004$), and refeeding days ($p = 0.005$) compared with control days. Resting time did not differ between starving and refeeding days ($p = 0.999$). The differences in the time spent grooming were also significantly different between the control period and starving days (lower when starving: $p = 0.016$), but not different between starving and refeeding days or between control and refeeding days. Time spent feeding was not significantly increased on the refeeding days compared with control days ($p = 0.41$) (Fig. 7).

3.2.4. Circulating leptin

Circulating leptin levels were related to body weight (ANCOVA: $F_{1,11} = 5.428$; $p < 0.04$), body fat content (ANCOVA: $F_{1,11} = 14.171$; $p < 0.003$) and independently treatment (ANCOVA: $F_{2,11} = 6.303$; $p < 0.015$) (Fig. 8). Levels of circulating leptin for the control group (6.2 ± 2.6 ng/ml) were significantly lower than for animals exposed to the starvation treatment (10.5 ± 1.8 ng/ml).

4. Discussion

4.1. Predation risk

According to the predation–starvation trade-off hypothesis, under increased risk of predation wood mice were predicted to decrease their fat reserves [12], thus decreasing their body weight. Consistent with this prediction, when mice were exposed to owl calls at night they significantly reduced their body weight relative to mice kept in silence. However, contrasting our expectations the mice also showed a significant reduction in body weight when exposed at night to calls of a non-predatory bird (blackbird).

Moreover, when exposed to either owl or blackbird sounds in the daytime, they did not reduce their body weight. The different effects of the sounds broadcasted during daytime and nighttime, are possibly associated with the animal activity periods. Wood mice are mostly active during the night [23,30]. Diurnal activity has been documented rarely and usually associated with the breeding season. Birds which naturally are active at night, where these mice live, are mostly potential predators. The mice may therefore have evolved to respond to any bird calling at night, and in contrast have similarly evolved to ignore

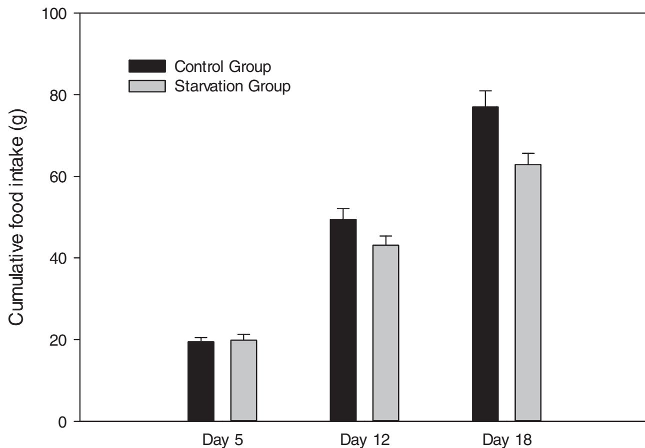

Fig. 6. Cumulative food intake (g) during the exposure to the stochastic starvation treatment. Day 5 (feeding day), day 12 (after two starving events), and day 18 (after four starving events).

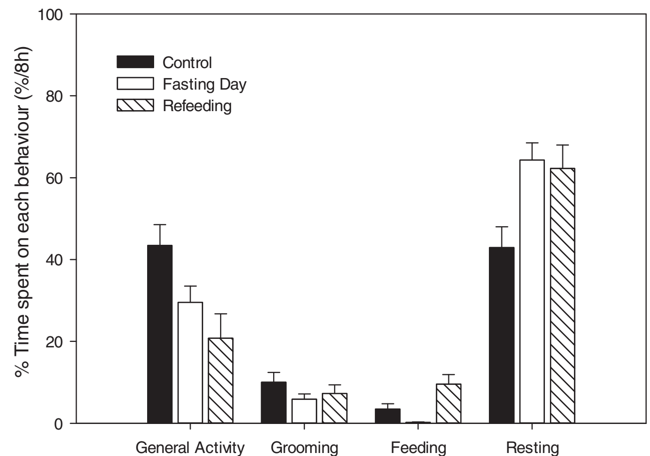

Fig. 7. Effects of the stochastic starvation treatment on the time spent in different behaviour categories (general activity, grooming, feeding and resting), on the starvation days and refeeding days.

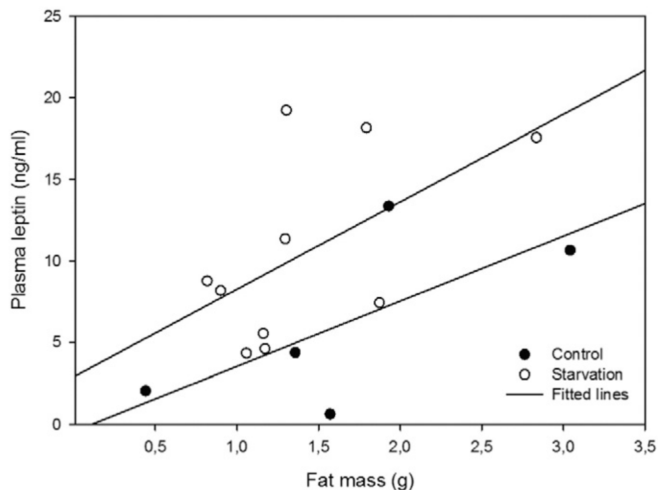

Fig. 8. Variation of plasma leptin levels (ng/ml) against body fat (g) for the control group and starved animals, measured on the final day of the study.

any bird calling in daytime. An alternative explanation was that during the day the mice were asleep and did not hear the calls, but at night they were awake and were stressed by any noise causing them to reduce intake and lose weight. However, this interpretation was at odds with the corticosterone data (see below) which clearly indicated that the mice could hear the calls in the daytime and if anything were more stressed by them that hearing calls at night. They just did not translate this stress into altered food intake or body weights.

These data suggest a generalisation of the response and the categorisation of the stimulus (reviewed by Shettleworth [31]). Large investments in predation avoidance may compromise other fitness components, such as reproduction [32], and the mechanism of discriminating traits may include an intensive training and learning process [33, 34]; therefore generalisation may be a mechanism of saving resources. In addition, with respect to predation stimuli, the opportunity for a learning process to occur may be limited, given that predator attacks are often fatal [35]. Hence most responses to predators may be innate, as has been demonstrated for several taxa [36]. According to the 'Predator Recognition Continuum Hypothesis' [37], a combination of innate and learned recognition mechanisms is probably involved. Kindermann [38] has demonstrated that predator-naïve rodents (mice, rats, and gerbils) do not discriminate their respond towards auditory cues of predator and non-predator birds. Since the mice in this experiment were wild caught from sites where all three of the bird species that we used are found, it is possible that they had been previously exposed to these calls in the wild before the experiments began, this factor was not controlled, hence we cannot rule out either a naïve or learned response to calls occurring at night.

The body weight responses of the mice suggested that mice were unable to discriminate between the owl calls and the blackbird calls, and instead reacted only to the cue of generalised bird calls, combined with the time of day that they were played. The data from the corticosterone assays supports the hypothesis that the mice were unable to distinguish between broadcasted sounds of different species, since the corticosterone levels were equally elevated during the diurnal period, independent of the type of bird call, and for exposure to calls at night were also not different between the sources of the calls. In an earlier study, faecal corticosterone levels in bank voles (*Myodes glareolus*) were elevated in response to weasel (*Mustela nivalis*) faeces and this was presumed to be part of the mechanism underpinning the reduction in body weight of these small rodents in response to predation risk [39]. Similarly rats showed elevated corticosterone in blood when exposed to a live cat (*Felis catus*) compared to a stuffed model [40], rabbits (*Oryctolagus cuniculus*) showed elevated corticosterone in blood when exposed to fox (*Vulpes vulpes*) as opposed to sheep (*Ovis aries*) faeces [41] and domestic mice had higher levels of cortisol when exposed to owl sounds compared to silence or human voice sounds [42]. Since the faecal corticosterone in the wood mice studied here was not elevated in the groups that lost weight, relative to the control mice that did not lose weight, this suggests that elevated corticosterone was not part of the mechanism underlying the altered body weight changes reported here. Furthermore, lag time between hormonal levels in the blood, and the signal on faeces is species-specific and highly dependent on animal gut function [43,44] which, as shown by Touma [45] is variable across the day. Therefore we consider that our method of perceived risk assessment may have underestimated the stress induced by the predation treatments, given that the levels of metabolites measured were highly affected by the circadian cycle. It is unlikely that reduced corticosterone levels are due to acclimation to the testing procedure, predation defence is highly repeatable [46,47], and habituation is not likely to cause fear extinction [48].

The reduction in body weight was correlated with a reduction in food intake. There were no differences in dry mass absorption efficiency and no change in resting metabolic rate beyond that anticipated from the reduced body weight. Although the data that we collected cannot rule out a contribution by elevated physical activity levels, the main mechanism for enabling the negative energy balance, to effect the reduction in body weight, was to reduce food intake. This strategy makes sense because it is probably during foraging that wood mice are most susceptible to avian predation risks. Hence reducing food intake probably has the dual benefit of directly reducing predation risk, at the same time as forcing a negative energy balance which reduces body weight, thereby generating secondary anti-predation benefits. Indeed the main benefit of losing weight in terms of predation risk may be the reduced energy demands (Fig. 3) which reduce the required foraging time.

4.2. Starvation risk

Although the responses of the wood mice to predation risk were largely in line with the predictions of the predation–starvation trade-off model the responses to experimental elevations in starvation risk were largely at odds with the predictions. We anticipated that in response to intermittent and unpredictable starvation events the mice would increase their levels of stored fat, enabling them to cope with subsequent starvation events without the need to enable behavioural or physiological responses, but simply by drawing on stored fat reserves. In fact, when exposed to starvation the mice altered their behaviour significantly reducing the costly components of their behaviour (activity and grooming) and increasing the less costly elements (resting and sleeping). Once food became available again they did not substantially elevate their food intake to offset the shortfall in their intake but instead had only a modest or insignificant increase in intake, matched with a continued suppression of activity to get themselves back into positive energy balance and increase in weight. This strategy meant that it took 2–3 days to recover their body weight after the starvation events, and they did not show any elevation in body weight above that of the controls. This was mirrored by the fact that at the end of the 18 day experiment the starvation group actually had lower fat reserves on average than the control animals — the complete opposite of the predictions of the starvation–predation trade-off hypothesis.

There have been many previous studies of the responses of other small mammals to periods of calorie restriction (reviewed in [49]) or to every other day feeding protocols. The responses of the animals to these previous manipulations are not directly comparable to the data generated here, the key element of which was the stochastic nature of the food deprivation events. Nevertheless, three previous studies have concerned the responses of domestic strains of mice to stochastic food deprivation. Swiss mice increased their food intake dramatically, and decreased their energy expenditure, on days between 24 h starvation events [50]. However, overall after 4 weeks, similar to the findings

here, their overall body weight was decreased. However, the same research group found over 4 weeks of treatment, with 3 starvation and 4 non-starvation days per week, that the elevated food intake on the feeding days was sufficient to maintain the body weight both in the same mouse strain and in striped hamsters [51]. In C57Bl/6 mice, in response to stochastic intermittent starvation, there was also a significant increase in food intake on the days between starvation events, such that over a 42 day experiment the overall intake was not reduced in the intermittently starved group [52]. Since these latter mice also enabled behavioural (reduced activity) and physiological (reduced body temperature) responses to the manipulation, at some points during the experiment they elevated their fatness above that of the control group, in line with the starvation–predation model predictions.

The responses of the wood mice studied here differ substantially from these previous studies. There was virtually no compensatory response in food intake. Hence, body weight was restored predominantly by reduced expenditure (reduced activity). Consequently they ended up with lower, rather than elevated fat reserves. One potential reason for this response is that the mice were constrained in their alimentary tract capacity and unable to process greater levels of food intake [53–55]. We cannot eliminate this possibility that the absence of a food intake response was due to a physiological constraint. A second reason, however, is that wood mice may be acutely sensitive to predation risks, and choose not to increase their food intake levels on the refeeding days, because this would expose them to elevated predation risk. Separating between these explanations was beyond the scope of the current work.

Circulating leptin levels in intermittently starved C57BL/6 mice were dependent only on the levels of body fatness [52]. This is consistent with many studies showing a relation of leptin to stored fat [56]. In wood mice the levels of leptin also were dependent on fatness, but in contrast to previous work were independently elevated among the mice that had been intermittently starved. This response was unexpected because reductions in food intake, for example, during caloric restriction, generally lead to reductions in circulating leptin levels which forms a primary stimulus to overconsume when released from restriction [5,57–59]. The fact that wood mice did not show this response to intermittent starvation in their leptin levels, is consistent with the fact they also did not show post-starvation hyperphagia. Understanding the mechanisms by which these mice elevate leptin levels after food restriction, and hence avoid post-restriction hyperphagia, may be important because in humans the reduced levels of leptin when dieting are presumed to be part of the primary response causing people to break their diets [60,61].

5. Conclusions

The current data provide limited support for the starvation–predation trade-off model for understanding levels of stored body fat/body weight. Wood mice responded in a manner that was complex and not entirely as expected, but could be interpreted as consistent with the model if it is assumed that their generalised response to calls at night was a generalisation to the predation risk of any bird in the wild that calls at night. However, when faced with elevated starvation they did not show the anticipated responses. This may in part be because these mice are substantially more susceptible to predation than to starvation, and their potential responses to starvation were compromised by the need to maintain a low level of predation risk.

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