

Body weight change as a measure of stress: a practical test

GW McLaren*, F Mathews, R Fell, M Gelling and DW Macdonald

Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

* Contact for correspondence and requests for reprints: graeme.mclaren@zoo.ox.ac.uk

Abstract

We report on the efficacy of body weight change as a measure of trapping and handling stress in two species of wild small mammal: bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*). We tested two hypotheses: (1) that weight change after capture and handling is related to the intensity of the trapping and handling regime, and (2) that weight change after an intensive handling regime is related to an individual's current pattern of energy expenditure. Trapped wood mice that were subjected to intensive handling (intensive stressor) lost more weight than did animals that were handled minimally (less intensive stressor), but this was not the case for bank voles. Patterns and factors related to body weight change in response to intensive handling also differed between the two species: heavier and non-breeding bank voles were more likely to lose weight, but this was not true for wood mice, and none of the factors we measured was found to affect weight loss in this species. Our results were broadly consistent with the predictions of the biological cost hypothesis. We discuss the limitations and benefits of weight loss as a measure of stress.

Keywords: animal welfare, body weight, handling, small mammals, stress, trapping

Introduction

Wild mammals are likely to show acute stress responses when they are captured and handled (eg Kenagy & Place 2000; Millspaugh *et al* 2000; Read *et al* 2000; Engelhard *et al* 2002). The energetic cost of mounting the stress response depends upon the frequency, duration and intensity of the stressor (Laugero & Moberg 2000; Moberg 2000). For captured wild mammals, the energetic cost of the stress response will therefore depend upon capture frequency and methodology. Estimating the biological cost imposed by capture and handling is important since mounting a costly stress response could affect an animal's future ability to survive normally in the wild. For example, female rhino subjected to repeated immobilisation suffer from reduced fertility (Alibhai *et al* 2001).

To meet the energetic cost of mounting a stress response, an individual must either use stored biological reserves, or, if these are insufficient, it must divert resources that would otherwise be used for other bodily functions (Moberg 2000). This model of stress predicts that the welfare implications of a given stressor will differ between individuals, depending not only on the level of their stored reserves, but also on their current pattern of energy expenditure. Thus, in mammals, the cost of mounting a stress response may have increased welfare implications during energetically costly periods such as growth and lactation, or if the individual is suffering from parasites or other diseases.

Biological energy reserves are usually stored as fat, and the biological cost model (Moberg 2000) predicts that stress should cause body weight loss as fat and other energy stores are utilised. For example, adult rats subjected to a moderate stressor of 3 h restraint for three consecutive days, suffer a reduction in body weight, which can consist of both lean and fat tissue, and in addition reduce their food intake (Harris *et al* 1998; Zhou *et al* 1999). Harris *et al* (2002) demonstrated that there were three stages to weight loss in response to stress: 1) a period of weight loss during stress; 2) a period of reduced food intake following the end of stress; and 3) an extended period of normal food intake but reduced body weight. The mechanisms responsible for these observed patterns are complex, and probably involve interactions between stress-related hormones and other hormones that affect food intake, including growth hormone and prolactin (Harris *et al* 2002). Furthermore, feeding responses to stress are not always straightforward: in rats, stress produced by pinching the tail has been shown to cause satiated animals to eat and to display oral stereotypies (Hawkins *et al* 1992).

Nevertheless, body weight change is a potentially useful and simple measure of the cost of capture and handling stress in wild mammals, particularly in situations where animals are likely to be recaptured (eg see Tuytens *et al* 2002) and when the animal's weight can be accurately and easily measured. However, since body weight change in response to stress will depend on the animal's energy

reserves and current pattern of energy expenditure, which may be affected by growth, reproductive investment and disease status, these must be measured before body weight changes can be fully interpreted. Fortunately, age (adult/juvenile), breeding and disease status can be recorded easily in most mammal species.

In this paper we examine the patterns of, and factors affecting, overnight body weight change in response to capture and handling in two species of wild mammal: bank voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*). We chose a two-stage approach to allow the two major components of the biological cost hypothesis to be tested independently: first, we determine whether weight loss is related to the intensity of the capture and handling stressor; second, we determine whether body weight change is related to the animal's current pattern of energy expenditure.

Methods

Study sites and trapping regime

The study sites were two commercial dairy farms in south Wales, UK. Each experiment was carried out on a separate farm. Small mammals were trapped overnight in Longworth live-traps placed along hedgerows. Traps were baited with approximately 14 g of 'Josie' rabbit food (Delta Pet Foods Ltd, Llandovery, Carmarthenshire, UK), and apple (approx 5 g) as a source of food and water. Laboratory trials had revealed that this bait mix was readily consumed by both species (McLaren unpublished data). The amount of bait provided meant that food was effectively available *ad libitum* during the period in the trap. Traps were checked each morning between 0730–0800h.

Experiment 1: minimal versus intensive handling

For this experiment, animals were caught during their summer breeding period (June–August) and were subjected to one of two treatments at random: (1) minimal handling (minimal stressor), and (2) intensive handling (intensive stressor), which involved sampling for an on-going wildlife disease study (details below). The minimal handling regime was designed to be less stressful than intensive handling, and consisted of removal from the trap, identification of species and sex, fur clipping, weighing to the nearest 0.1 g on a digital balance and release. The intensive handling regime consisted of removal from the trap, anaesthesia and sampling for a wildlife health-monitoring project. When captured for the first time, animals were anaesthetised using a mixture of oxygen and isoflurane (Mathews *et al* 2002). After identification of species, sex and reproductive status (based on testes status in males and indications of mating or pregnancy in females [Gurnell & Flowerdew 1990]), they were weighed to the nearest 0.1 g. Samples (blood spots for DNA analysis, tracheal aspirate and fur) were taken as part of a separate study. Some juveniles were considered to be too small for full sampling, and as a result we only included adult animals (15 g and heavier) in our analyses. The animals were also searched for parasites. Fleas were the most common parasite encountered, and animals were classified either as having or not having fleas, and also as

having or not having other visible parasites, which included mites and ticks. Hydration was classified as normal or poor based on the speed at which a pinch of skin receded following release: animals whose skin was slow to return to normal were classified as poorly hydrated. Each animal was given a subcutaneous injection of Hartman's solution, a mixture of water and salts (Animalcare Ltd, Dunnington, York, UK), at a dosage of 0.04 ml/g body weight. This is a re-hydration solution given to encourage the animals to urinate (urine was collected for a separate study). Animals were also given an individual fur clip mark that could later be used to identify them if they were recaptured.

After handling (minimal or intensive), all animals were placed in plastic holding pots designed to carry small animals, and each was given a fresh piece of apple (approx 5 g) and then kept in a dark, quiet room and returned to its site of capture at 1400h. The time spent in captivity (after a night in the trap) was 6 h. Animals that were recaptured 24 h later were re-weighed and released, and changes in their body weights were recorded. This work was carried out under Home Office Licence PPL 30/1826.

Since the ability to accurately determine hydration and parasite status was greatly enhanced by observation of the animals under anaesthesia, for this part of the study we did not attempt to include these parameters in our models predicting overnight weight loss. Furthermore, since almost all of the adult animals were in breeding condition, and since observation under anaesthesia may improve the accuracy of breeding condition assessment, we restricted our analysis to the effects of treatment, species and sex on weight loss. The effects of breeding, hydration and parasite status were examined in the second experiment.

Experiment 2: factors affecting responses to handling

Our second experiment involved carrying out a detailed survey of overnight weight change in individuals that had undergone our wildlife health-sampling regime (intensive handling, as described above). Animals for this study were trapped between January–March 2002, and hence had just over-wintered and represented a group of adult individuals that were either non-breeding or just at the start of their breeding season. Animals were trapped as outlined above, and those that were recaptured 24 h after release were re-weighed and released.

Statistical analyses

To examine the factors affecting body weight change (treatment, initial weight, species, sex and treatment*species interaction) we used a univariate General Linear Model (GLM). For the results of the GLM, we present *F* and *P*-values, and for continuous variables (weight change and % weight change) we present *B*, which indicates the strength and direction of the relationship: positive values indicate that as the parameter increases weight is gained and vice versa. We also used binary logistic regression to determine if any factors affected the incidence of recapture, and paired *t*-tests to look at weight change in

Table 1 Patterns of weight loss in wood mice and bank voles in response to minimal and intensive handling treatments. * Indicates a significant ($P < 0.05$) change in body weight.

Handling treatment	Mean weight change \pm SD	
	Wood mice	Bank voles
Minimal	0.18 \pm 0.96 g	0.18 \pm 0.74 g
Intensive	-0.46 \pm 1.17 g*	0.12 \pm 0.87 g

recaptured individuals. All analyses were carried out using SPSS for Windows, version 11.0.

Results

Experiment 1: minimal versus intensive handling

For this experiment, 113 animals were caught and recaptured the next day, of which 54 (wood mice $n = 27$; bank voles $n = 27$) were minimally handled, and 59 (wood mice $n = 36$; bank voles $n = 23$) were intensively handled. Patterns of overnight weight loss in the two species in response to the two handling treatments are given in Table 1. The GLM analysis indicated that treatment had a significant effect on % weight loss ($F_{1,101} = 6.3$; $P = 0.01$). There were no effects of sex ($F_{1,101} = 1.6$; $P = 0.20$), initial weight ($F_{1,101} = 1.2$; $P = 0.29$) or species ($F_{1,101} = 0.8$; $P = 0.37$), but there was evidence of a treatment*species interaction effect ($F_{1,101} = 3.8$; $P = 0.06$). Paired t -tests indicated that wood mice lost weight in response to intensive handling (mean weight loss = 0.46 g; standard deviation [SD] = 1.17; $t = 2.4$; $P = 0.03$), but not in response to minimal handling ($t = -0.64$; $P = 0.52$). Bank voles did not lose weight in response to either stressor (intensive handling: $t = -0.05$; $P = 0.60$ and minimal handling: $t = -1.3$; $P = 0.22$).

Experiment 2: factors affecting responses to intensive handling

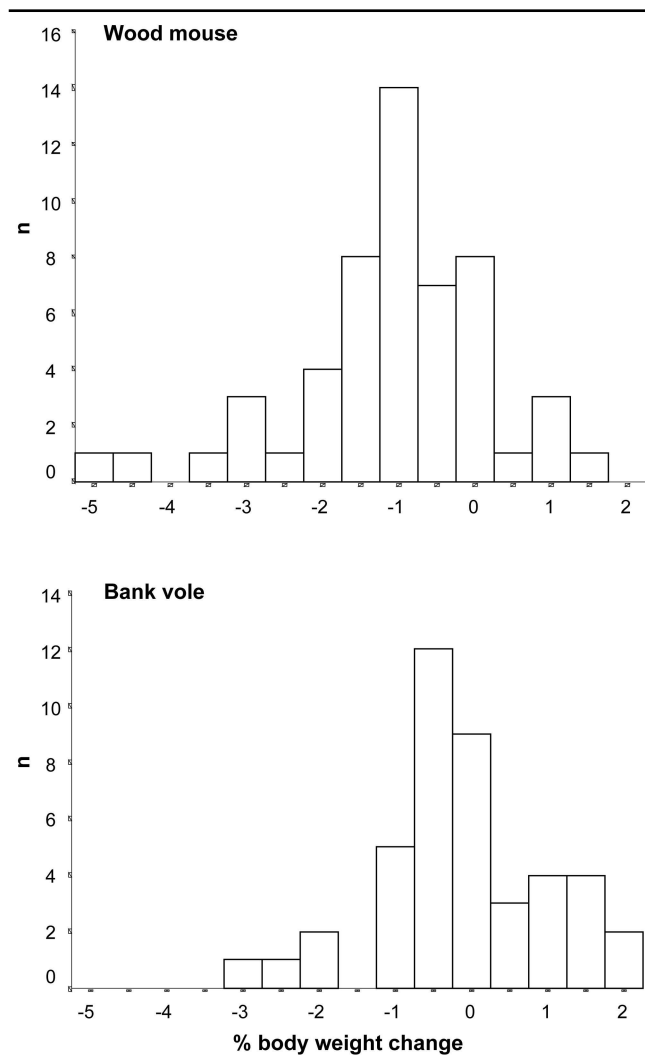
185 individuals were caught during this study; 121 (65%) of which were recaptured at some point and 96 (52%) of which were caught on consecutive days and were used in the analysis. Of these, 53 were wood mice (male: 22; female: 31) and 43 were bank voles (male: 20; female: 23). Initial weights (mean \pm SD) of these animals were: bank vole males: 22.5 \pm 3.3 g and females: 19.9 \pm 4.6 g; wood mouse males: 26.2 \pm 2.6 g and females: 22.0 \pm 3.5 g. Further details of these animals are given in Table 2. Since we had detailed observations under anaesthesia of each of these animals, we took the opportunity to look for factors that affected the chances of recapture. We did not find any effects of species, sex, breeding condition, hydration, body length, initial weight or parasite status on the chances of an animal being recaptured or consecutively recaptured (ie recaptured 24 h later) (binary logistic regression, all $P > 0.1$).

Because of the timing of the trapping, our captures were restricted to adult animals consisting of a mixture of non-breeding and breeding individuals (Table 2). Histograms of % body weight change between the first and second capture

Table 2 Details of the hydration, breeding and parasite status of the 96 animals that were caught and recaptured 24 h later (consecutively recaptured), and which were used to examine the factors affecting responses to handling (Experiment 2).

Factor	Wood mice		Bank voles	
	Yes	No	Yes	No
Normal hydration	38	15	17	26
Breeding	36	17	28	15
Fleas	22	31	27	16
Other parasites	4	49	20	23

Figure 1



Histograms of % body weight change after capture and intensive handling in wood mice ($n = 53$) and bank voles ($n = 43$).

are shown in Figure 1, and these indicate that there were species-specific differences in weight loss, with the majority of wood mice losing weight, in contrast with the more balanced pattern of weight loss and gain in bank voles. Paired t -tests indicated that in wood mice there was a significant change in body weight between Day 1 and Day 2

($t = 4.6$; $P < 0.001$), with mean body weight being reduced by 0.86 g (SD = 1.3 g). A total of 30 wood mice were captured for three consecutive days, and mean body weight between capture Day 2 and Day 3 was not significantly different in these individuals ($t = -0.38$; $P = 0.7$). In contrast, there was no significant overall change in body weight between Day 1 and Day 2 in bank voles ($t = -0.40$; $P = 0.7$), although 25 individuals caught on three consecutive days showed a drop in body weight between Day 2 and Day 3 (mean reduction \pm SD: 0.56 ± 1.2 g; $P = 0.02$).

Our initial GLM model also indicated that there were significant species effects on the % body weight change between first and second captures ($F_{1,92} = 13.6$; $P < 0.001$), but no effect of sex ($F_{1,92} = 0.26$; $P = 0.61$) and no interaction between sex and species ($F_{1,92} = 0.02$; $P = 0.89$). To examine body weight loss between the first and second day's capture, we therefore chose to build individual models for each species but with the sexes combined. In bank voles, initial weight and breeding status were significant predictors of % weight change: heavier voles were more likely to lose weight, as were non-breeding animals (initial weight: B (standard error) = -0.73 g (0.25); $F_{1,37} = 8.84$; $P = 0.005$, breeding status: $F_{1,37} = 8.30$; $P = 0.007$). These relationships were maintained when we examined actual weight change (rather than %) (initial weight: B (standard error) = -0.123 g (0.05); $F_{1,37} = 7.24$; $P = 0.011$, breeding status: $F_{1,36} = 7.36$; $P = 0.01$). However, these two variables were not independent since breeding bank voles had significantly greater initial weights than did non-breeders ($t = -2.3$; $P = 0.03$). No significant relationships were found for wood mice.

Discussion

The two main predictions of the biological cost hypothesis were broadly supported by the results of the two experiments: the handling experiment revealed that intensive handling resulted in a mean decrease in body weight in recaptured wood mice, but minimal handling did not. Although bank voles appeared to be more robust in terms of maintaining body weight in response to handling stress, there were patterns in weight loss in this species that were related to initial body weight and reproductive status.

In order to be a relevant and meaningful measure of the stress response, body weight change must reflect the impact of a stressful event upon the individual, and must be unaffected by other activities that are carried out by the investigators. Clearly, our results would be invalid if there was evidence that the animals were simply starved, or that one of our procedures had caused weight loss. However, animals were initially attracted into the trap by the presence of food and whilst in the trap food was available *ad libitum*. The type of food we provided is sufficient to maintain captive colonies of wood mice and bank voles (McLaren unpublished data), and food restriction whilst the animal was in the trap would therefore appear unlikely. This is further supported by the observation that the mean body weight of trapped and minimally handled animals of both species increased. Our procedures were also unlikely to

cause weight loss *per se*: animals were re-hydrated and were never without a food source except when actually under anaesthesia. Furthermore, we can think of no reason why our procedures alone would cause species-specific patterns of weight loss.

The analysis of weight loss in both experiments revealed species differences, and, in response to stress, wood mice were more likely to lose weight than were bank voles. For bank voles, non-breeding animals and those of high initial body weight lost most weight in response to our procedures. Therefore, for bank voles, effects of stress were related to the resources available to the animal (represented by initial weight) and to its current pattern of energy expenditure (represented by breeding status). In this instance, body weight change reflected the energy gained from the food that we provided and the energetic cost of mounting a stress response, and it therefore appears that for lighter and breeding bank voles the energy gained in terms of food eaten outweighed the energy invested in the stress response. A hypothesis to explain our observations is that, in response to stress, heavier and non-breeding bank voles had greater utilisable fat stores than did lighter or breeding individuals, and that the utilisation of this fat resulted in them losing a greater % of their initial body weight.

In contrast, the pattern of weight loss in wood mice was unrelated to initial weight, breeding status or any of the other parameters we measured. The different weight loss patterns observed in the two species may reflect their different energetic patterns and feeding strategies (reviewed by Grodzinski 1985). The diet of bank voles is higher in fibre and less digestible than that of wood mice, and bank voles have a relatively higher daily energy budget and lower production efficiency (Grodzinski 1985). This could have consequences for food intake after stress, and suggests that wood mice, with their more digestible diet and relatively lower energy demands, would have more scope to sacrifice food intake to increase survival. This could explain the observation that most wood mice lost weight between Days 1 and 2, which was not apparently related to energetic status. Therefore, we hypothesise that stress-induced weight loss is dependent not only upon the energetic status of the individual, but also upon the feeding and energetic strategies of the species.

As a measure of the stress response in wild mammals, body weight change has drawbacks. First, unless there are non-invasive means of weighing the animal, it must be recaptured in order to be re-weighed, thereby subjecting it to further stress. Second, the absence of weight loss after a stressful event cannot be used to indicate that the stressor did not impose a significant biological cost. For example, in rats, stress-related body weight loss is dependent upon sex and strain (Faraday 2002), and levels of restraint stress sufficient to decrease body weight in male rats of two strain types had no effect on the body weights of females of these strains, even though the females of one of the strains exhibited depression-like behaviour (Faraday 2002).

Conclusions and animal welfare implications

Body weight responses are an appropriate measure of capture and handling stress when, as in our case, animals are likely to be recaptured as part of the normal trapping procedure. Study animals should not be subjected to frequently repeated trapping and handling procedures that cause weight loss, although the absence of short-term weight loss in wild mammals after a stressful handling event cannot be interpreted as evidence that the event has no significant biological cost.

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