

Huddling in groups leads to daily energy savings in free-living African Four-Striped Grass Mice, *Rhabdomys pumilio*

M. SCANTLEBURY,*† N. C. BENNETT,* J. R. SPEAKMAN,‡ N. PILLAY§
and C. SCHRADIN§

*Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa, †Aberdeen Centre for Energy Regulation and Obesity (ACERO), University of Aberdeen, School of Biological Sciences, Tillydrone Ave. Aberdeen AB24 2TZ, UK, and §School of Animal, Plant and Environmental Sciences, Ecophysiological Studies Research Group, University of the Witwatersrand, Private Bag 3, Wits 2050, Johannesburg, South Africa

Summary

1. Free-living animals make complex decisions associated with optimizing energy and nutrient intake. In environments where ambient temperatures fall below the thermoneutral zone, homeotherms must choose whether or not to forage, how long and what to forage for, and whether or not to perform activities that conserve energy.

2. Huddling in groups has long been thought of as a possible means of conserving energy. Laboratory studies have shown that at low ambient temperatures individuals in groups expend less energy than individuals by themselves. However, studies have yet to demonstrate that thermoregulatory savings can have an impact on the overall daily energy expenditure (DEE) of free-living animals.

3. Here we show that, in the laboratory, African Four-Striped Grass Mice (*Rhabdomys pumilio*) expend less energy per individual in large groups than smaller groups. We also show that when free-living groups were experimentally reduced to one-half of their original size, DEE and water turnover increased by 19% and 37%, respectively.

4. The magnitudes of the reduction in free-living DEE were comparable with calculated energy savings from the laboratory. One of the reasons why this species may sometimes occur in groups is that energetic benefits can be gained through huddling in habitats in which food and water are scarce.

Key-words: Daily energy expenditure, doubly labelled water, resting metabolic rate, sociality, thermoregulation

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Introduction

An animal's fitness depends on its ability to partition time and energy among competing behavioural and physiological demands in a way that optimizes benefits relative to costs (Schoener 1971; McNab 1980, 2002). In an environment in which ambient temperatures fall below the thermoneutral zone, homeotherms must make the choice of whether and how long to acquire energy while being exposed to external climatic variation, or whether and how long to perform activities that conserve energy, such as seeking refuge in a nest (Bozinovic *et al.* 2000). The problem is especially acute for non-hibernating small mammals because they lose heat rapidly and need to consume large

amounts of food relative to their body mass (Canals, Rosenmann & Bozinovic 1989; Canals, Rosenmann & Bozinovic 1997). Behavioural mechanisms to conserve energy can be expected to occur whenever ambient temperatures fall below the thermoneutral zone (Vickery & Millar 1984; Contreras 1984). Animals may, for example, return to a central place such as a nest and reduce the energy cost of foraging (Charnov 1976; Orians & Pearson 1979), they may bask in the sunshine (Geiser, Goodship & Pavey 2002), they may allow body temperature to fall (Geiser 2004) or they may huddle in groups during periods of cold (Kaufman *et al.* 2003).

Laboratory studies have shown that huddling can reduce both energy expenditure and water use in birds and mammals (e.g. Hayes, Speakman & Racey 1992; Kaufman *et al.* 2003). Minimization of thermoregulatory costs and water loss might then be one reason that

animals aggregate (Madison 1984; Andrews & Belknap 1986; Berteaux *et al.* 1996; Canals *et al.* 1998). There is strong evidence that even animals that are normally solitary can aggregate for the sole purposes of thermoregulatory savings (e.g. Fedyk 1971; Rhind 2003). However, few studies have yet attempted to measure the energy savings due to huddling in free-living small mammals (although see Berteaux *et al.* 1996; Kenagy & Pearson 2000). To our knowledge no study has shown that the energetic effects of huddling have an overall impact on the daily averaged energy expenditure (DEE). Demonstration of such an effect would provide strong support for thermoregulation as a factor leading to the evolution of group living (Beauchamp 1999; Schradin & Pillay 2005a).

African Four-Striped Grass Mice *Rhabdomys pumilio* have a wide distribution across southern Africa and are found in arid habitats as well as grasslands (Skinner & Chimimba 2005). They are diurnal and may be either solitary or group living. They may also dig their own burrows, inhabit unoccupied burrows of other animals, or construct nests above ground (e.g. made of hay). In the succulent karoo, a semiarid region in the north-west of South Africa, *R. pumilio* constructs its nests under shrubs above ground and lives in social groups (Schradin & Pillay 2004). Groups typically consist of one breeding male, two to four breeding females and their philopatric offspring of both sexes (Schradin & Pillay 2004). Although mice of one group share one nest and one territory (which they defend against mice of other groups), they forage alone during the day (Schradin 2004; Schradin & Pillay 2004). Benefits of living in groups are suggested to include communal care of offspring and reduction of risk of predation at the nest (through increased vigilance) (Schradin 2006a). However, there may also be other benefits of living in groups, such as the thermoregulatory benefits of huddling, especially on cold nights. Although the benefits of huddling are likely to be beneficial throughout the year (temperatures may vary from below zero to over 25 °C during the winter and from +5 °C to over 40 °C during the summer: M. Scantlebury, personal observation), they are presumably greatest during the winter. In addition, since striped mice in the succulent karoo construct their nests above ground, they may be especially susceptible to low night-time temperatures. Therefore, they have to compromise between the ability to dissipate heat during the day when it is hot, and reduce thermoregulatory costs at night when it is cold. Moreover, because they live in an environment that is seasonally restricted in both water and food, any behavioural or physiological mechanism that conserves water and energy is advantageous (Berteaux *et al.* 1996). Hence, the possible benefits of group living may include the ability to save water and energy during the night.

In the current study we aimed to determine: (1) whether individual Four-Striped Grass Mice expend

less energy in the laboratory as group size increases and whether these energy savings increase when ambient temperature decreases; and (2) what (if any) are the effects of group size on the DEE and water turnover (WTO) of free-living mice, by experimentally manipulating free-living group size.

Materials and methods

STUDY SITE AND ANIMALS

Fieldwork was conducted in the Goegap Nature Reserve, Northern Cape Province, South Africa (29°41'712'S, 18°01'558'E). The study area consists of approximately 10 ha of succulent karoo – open semidesert (Acocks 1988; Cowling, Esler & Rundel 1999). The vegetation comprises mainly *Zygophyllum retrofractum* and *Lycium cinerum* shrubs, which form small islands separated by open sandy areas that are interspersed with succulents and ephemerals (in spring) (Rösch 2001). The study took place during the winter, before the onset of the breeding season (Schradin & Pillay 2005b) and all Four-Striped Grass Mice studied were non-reproductive adults. Nine study groups were used (mean group size 4.1 ± 1.6 (SD) mice, range 2–6), which are part of an ongoing study (since 2001). Mice are regularly trapped and individuals from each group are fitted with radio-collars (Schradin & Pillay 2005c); home ranges and sleeping associations are therefore readily determined (Schradin & Pillay 2004; Schradin & Pillay 2005c). Ear tags (8 mm, 0.12 g; National Band and Tag Co., Newport, KY, USA) were used to permanently mark mice. For ease of identification in the field, a number was also written on the side of each mouse with black hair dye (Inecto Rapid, Pinetown, South Africa).

CAPTIVE MICE

Captive Four-Striped Grass Mice (consisting of the F3 descendents of wild-caught individuals from the same population used for the field study) were used. They were kept under natural weather conditions at the research station next to the field site (Schradin & Pillay 2005a). They were housed in $40 \times 12 \times 25$ cm³ (l × h × w) Laboratory-o-tec^R cages (Halfway House, South Africa). The cages were protected from direct sun and rain but were otherwise exposed to external ambient conditions. Hay and tissue paper were provided as bedding and nesting material; water was provided *ad libitum*. Four grams of seed mix was provided to each mouse, each day.

OXYGEN CONSUMPTION ($\dot{V}O_2$) IN CAPTIVE MICE AT DIFFERENT TEMPERATURES AND FOR DIFFERENT GROUP SIZES

Oxygen consumption values in ml O₂ h⁻¹ ($\dot{V}O_2$) were determined for different group sizes of captive mice at

different temperatures. Temperatures of 5, 10, 15, 20, 25 and 30 °C were used with different individual groups of mice of group sizes one, two, three, four, four, six and eight mice. The sexes for these groups were: one male; one male and one female; two males and one female; four females; four females; four males and two females; and six males and two females, respectively. The temperatures selected spanned a large part of the natural daily variation in ambient temperature experienced by the mice, during the winter, at the time of study (Cowling *et al.* 1999): 5 °C is approximately the minimal expected nest temperature (C. Schradin and N. Pillay, unpublished data) and 30 °C is just below the thermoneutral zone (Haim & Fourie 1980), beyond which thermoregulatory benefits of huddling cease. Measurements took place during the night (approx. 20.00–03.00 h) when mice would normally be sleeping. We used a dim red light (<50 lux), which allowed us to work during the night but did not disturb the mice (Haim & Rubal 1994). Groups were placed in the respirometry chamber for at least 30 min before any measurements took place in order for them to settle down (Speakman *et al.* 2003). After this initial period, beginning at the lowest temperature (5 °C), we took measurements of oxygen consumption every 30 s for at least 20 min (or longer if the animals did not settle). We then increased the temperature of the water bath by 5 °C (i.e. to 10 °C from 5 °C) and allowed the mice to settle for a further 30 min. Measurements were then again taken every 30 s for another 20 min. These procedures were repeated for all the required temperatures. At each temperature, the mean of the lowest 10 readings of oxygen consumption ($\text{ml O}_2 \text{ h}^{-1}$) was taken, when animals were seen to be at rest (Bennett *et al.* 1992). An open circuit respirometry system (Depocas & Hart 1957; Hill 1972) was used in which a metabolic chamber (15710 cm^3) was immersed in a temperature-controlled water bath (Labotec, Lauda, Königshofen, Germany). The same chamber was used for all measurements (i.e. for all group sizes of mice and for all temperatures). Dried air was pumped into the chamber at a variable rate ($\text{min } 500 \text{ ml min}^{-1}$; $\text{max } 4000 \text{ ml min}^{-1}$) determined by the number of mice (we used approximately 500 ml min^{-1} for each mouse that was in the chamber) so that depressions in oxygen concentration were maintained at 0.25–0.4%. The air passed through approximately 4 m of copper coil that was submerged in the water before it entered the chamber. This ensured the temperature of air that entered the chamber was the same as the water bath. The flow of air into the chamber was controlled by a flow regulator (Omega FMA-A2310, Stamford, CT) placed upstream. Measurements of $\dot{V}\text{O}_2$ were taken using an oxygen analyser (S-2 A Applied Electrochemistry, AEI Technologies, Inc., Naperville, IL, USA). The analyser was calibrated to an upper value in dry air (20.95% O_2) prior to the measurement of each animal and to a lower value (0% O_2 in N_2 gas, Afrox, Germiston, South

Africa) prior to initial measurements. Results were corrected to standard temperature and pressure.

RESTING METABOLIC RATE (RMR) OF FREE-LIVING MICE

RMR was determined as the minimal oxygen consumption ($\text{ml O}_2 \text{ h}^{-1}$), using the apparatus described above for individual mice after they had been captured from the field (after the second recapture, see DLW methods below). The temperature of the water bath was maintained at $31 \pm 0.5 \text{ }^\circ\text{C}$, which is within the thermoneutral zone (Haim & Fourie 1980). Readings were taken when animals were seen to be at rest, after an initial hour in which they were allowed to settle in the respirometry chamber (Speakman *et al.* 2003). As before, measurements of oxygen consumption were taken every 30 s for 20 min and the mean of the lowest 10 readings of oxygen consumption ($\text{ml O}_2 \text{ h}^{-1}$) was taken, when animals were seen to be at rest (Bennett *et al.* 1992).

DAILY ENERGY EXPENDITURE (DEE) OF FREE-LIVING MICE

The daily energy expenditures (DEE, kJ day^{-1}) of nine adult mice (eight females and one male from nine different groups, with group sizes of two, three, four, four, four, four, six, six and six individuals) were measured using the doubly labelled water (DLW) technique (Lifson & McClintock 1966; Speakman 1997). One mouse per group was used. Because males were frequently fitted with radio-transmitters, but females were not, we decided to concentrate on measuring the DEE of females. This had the additional advantage of omitting intersexual variation in DEE. However, for one of the groups of mice we did not capture a female. Instead, we captured a male with a transmitter. Since the DEE of this one male did not differ statistically from that of all the other females ($P = 0.1$), we decided to include this one male in the analysis. On day 1 (the first day of capture), the animals were weighed ($\pm 0.1 \text{ g}$) and a 0.1-ml blood sample was obtained from the tail to estimate the background isotope enrichments of ^2H and ^{18}O . Blood samples were immediately heat sealed into 50- μl glass capillaries. Afterwards, a known mass of DLW [100 g 95% APE-enriched ^{18}O water (Rotem Industries Ltd, Beer Sheva, Israel) and 50 g 99.9% APE-enriched ^2H water (Isotec Inc. Miamisburg OH) mixed with 342 g $^1\text{H}_2^{16}\text{O}$] was administered (IP, 0.3 g/100 g body weight). Syringes were weighed before and after administration ($\pm 0.0001 \text{ g}$, Sartorius balance) to calculate the mass of DLW injected. Blood samples were taken after 1 h to estimate initial isotope enrichments. Mice were trapped 2–3 days later (Speakman & Racey 1988) to estimate isotope elimination rates. Half of the mice of each group were then temporarily removed and housed together in cages in the outdoor enclosure,

thus reducing group sizes in the field by half. The previously injected mice were then re-trapped 2–3 days later (i.e. 4–6 days post dose) to estimate isotope elimination rates for the same mice with reduced group sizes. We then used equation 7.17 of Speakman (1997) for small mammals to calculate DEE values at the reduced group sizes. We used the measured values of k_o and k_d (the oxygen and deuterium turnover rates, respectively, per day) from the mice with reduced group sizes and calculated the new N_d (the body water space calculated by deuterium dilution, moles) values on the assumption that the ratio of isotope pool size to body mass measured initially, remained constant for the duration of the experiment (the ‘percentage mass’ assumption: Speakman 1997). Capillaries that contained the blood samples were vacuum distilled (Nagy 1983) and water from the resulting distillate was used to produce CO_2 and H_2 (methods in Speakman 1997 for CO_2 and Speakman & Krol 2005 for H_2). The isotope ratios $^{18}\text{O}:^{16}\text{O}$ and $^2\text{H}:^1\text{H}$ were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom μG , Manchester, UK), prior to calculation of DEE (Lemen & Speakman 1997). Recaptured animals (i.e. those that had been recaptured twice) were taken back to a field laboratory where their RMR was immediately measured (see above). In addition to DEE and RMR, sustained metabolic scope (an independent index of how hard an animal is working; $\text{SusMS} = \text{DEE}/\text{RMR}$) was calculated for each animal (Drent & Daan 1980). Water turnover (WTO) values (ml day^{-1}) were calculated using the measured deuterium elimination rates (k_d) and deuterium dilution spaces (N_d) (Lifson & McClintock 1966; Nagy & Costa 1980) using the equation:

$$\text{WTO (ml day}^{-1}\text{)} = k_d N_d \times F, \quad \text{eqn 1}$$

where F is the fractionation factor of the isotope ($= 0.941$: Speakman 1997). Water economy index (WEI) values (ml kJ^{-1}) (Nagy & Peterson 1988) were calculated using the equation:

$$\text{WEE (ml kJ}^{-1}\text{)} = \text{WTO}/\text{DEE}. \quad \text{eqn 2}$$

STATISTICAL ANALYSIS

Generalized linear models (GLM) were used to test for the effects of group size on VO_2 in the captive mice as well as to test for the effects of experimental group size reduction on DEE, RMR, SusMS and WTO in free-living mice (McKenzie & Goldman 1998). Group mass and temperature were included as covariates in the former (i.e. for the laboratory mice) models, body mass and group size were as covariates in the latter (i.e. for the free-living mice). One model was fitted for each energy expenditure measure and all interaction terms were fitted in all models. Results are presented as means \pm standard deviations.

Results

EFFECTS OF HUDDLING IN CAPTIVE MICE

Captive mice weighed on average 48.7 ± 13.8 g. There was a significant interaction between group mass and temperature on VO_2 ($F_{1,38} = 36.69$, $P < 0.001$). VO_2 increased with decreasing temperature and this effect was smallest in the largest groups, i.e. individual mice consumed less oxygen in larger groups and the effect was especially apparent in larger group sizes (Fig. 1). The largest increase in VO_2 was found in one individual vs many (Fig. 1). To examine whether the significance of the overall relationship was in fact an artefact of the difference in VO_2 of one individual vs many, we removed the data from the one individual and repeated the analysis. We found that the effects of group mass and temperature on VO_2 remained significant even when the data from one individual were removed ($F_{1,32} = 16.69$, $P < 0.001$). Hence, we concluded that the effects of group size on temperature were consistent across the range of group sizes. The least-squares fitted regression,

$$\text{VO}_2 \text{ (per animal, ml O}_2 \text{ h}^{-1}\text{)} = 326 - 7.04 \times \text{temp (}^\circ\text{C)} - 15.0 \times \text{group size}, \quad \text{eqn 3}$$

explained 86.5% of the variation ($F_{2,39} = 124.77$, $P < 0.001$).

RESTING METABOLIC RATE (RMR) OF FREE-LIVING MICE

RMR was correlated with body mass ($F_{1,5} = 60.42$, $P < 0.001$) but not with free-living group size ($F_{1,5} = 0.49$, $P = 0.516$). The least-squares fitted regression,

$$\text{RMR (ml O}_2 \text{ h}^{-1}\text{)} = 10.5 + 1.54 \times \text{body mass (g)}, \quad \text{eqn 4}$$

explained 92.5% of the variation ($F_{1,6} = 74.47$, $P < 0.001$).

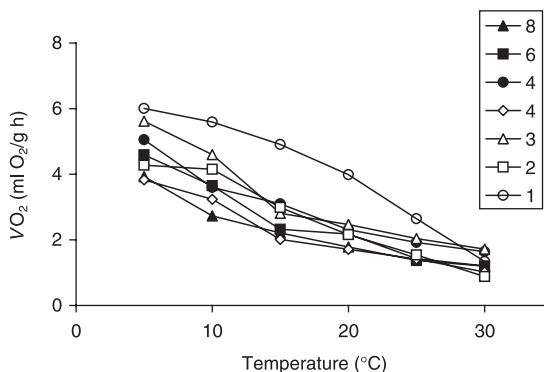


Fig. 1. Oxygen consumption (VO_2 , $\text{ml O}_2/\text{g h}$) against temperature ($^\circ\text{C}$) of *R. pumilio* for group sizes of one, two, three, four, four, six and eight individuals.

DAILY ENERGY EXPENDITURE (DEE) AND
SUSTAINED METABOLIC SCOPE (SUSMS) OF
FREE-LIVING MICE

Mice weighed on average 41.7 ± 8.9 g ($n = 9$) upon initial capture. There was a significant positive effect of body mass on DEE ($F_{1,6} = 6.14$, $P = 0.048$ and $F_{1,6} = 19.46$, $P = 0.005$ for control and reduced groups, respectively), but there was no effect of group size ($F_{1,6} = 2.51$, $P = 0.164$ and $F_{1,6} = 0.09$, $P = 0.779$ for control and reduced groups, respectively) on DEE. Therefore, it was not the case that mice in naturally smaller group sizes had significantly higher DEE values. DEE values increased by a mean of 19% (10.8 kJ day⁻¹) when group sizes were reduced by one-half (70.0 ± 18.1 and 59.1 ± 13.2 kJ day⁻¹, respectively, two-sample $t = 2.57$, $P = 0.03$, Fig. 2a). There was no effect of body mass or group size on the increase in DEE when groups were reduced ($F_{1,6} = 3.58$, $P = 0.107$ for body mass, $F_{1,6} = 1.78$, $P = 0.230$ for group size). Therefore, it was also not the case that the increase of DEE (upon reduction of group size) was correlated with original group size or the body mass of the measured individual. We were interested in whether we could detect any effects of natural variation in group size on DEE. Since including too many covariates in a model with small sample size diminishes statistical power (MacCallum, Browne & Sugawara 1996), we reanalysed the DEE data slightly differently by using each covariate (body mass

and group size) separately, using GLM analyses. In the control group, we found significant positive and negative effects for mass ($F_{1,7} = 11.31$, $P = 0.01$) and group size ($F_{1,7} = 5.84$; $P = 0.046$), respectively. By comparison, although there was a significant effect of mass when groups had been reduced ($F_{1,7} = 27.38$, $P = 0.001$), there was no significant effect of group size ($F_{1,7} = 3.84$, $P = 0.09$). These results suggest that there might have been effects of natural group size on DEE but that our sample size was too small ($n = 9$ groups) to detect significant differences when both covariates were included in the same model. SusMS averaged 1.41 ± 0.19 in natural group sizes. There were no effects of body mass or group size on SusMS ($F_{1,5} = 0.55$, $P = 0.49$ for body mass and $F_{1,5} = 2.42$, $P = 0.18$ for group size), or any significant change in SusMS when group size was reduced.

Assuming that mice gain the benefits of huddling for the duration that they are in the nest (*c.* 14 h day⁻¹ in the winter and 10.5 h day⁻¹ in the summer: C. Schradin, personal observation), we calculated the average expected increase in DEE for mice in the free-living group sizes that we measured using the derived relationship between temperature and group size on VO_2 (Equation 3). We calculated the difference in VO_2 for the range of free-living group sizes that were measured for DEE and for those that had been reduced by half. We then converted the difference between these two values of VO_2 to kJ day⁻¹ using a factor of 20.51 kJ l⁻¹ O₂ (Hardy 1972). As this figure would indicate the energy increase due to a reduction in group size for 24 h, we converted this into an equivalent increase in energy expenditure for a 14-h period, which was the length of time that mice were in their nests (huddling) at night. Using this method, the calculated average increase in DEE for the free-living mice for which group size had been reduced by one-half was 9.3 ± 3.0 kJ day⁻¹.

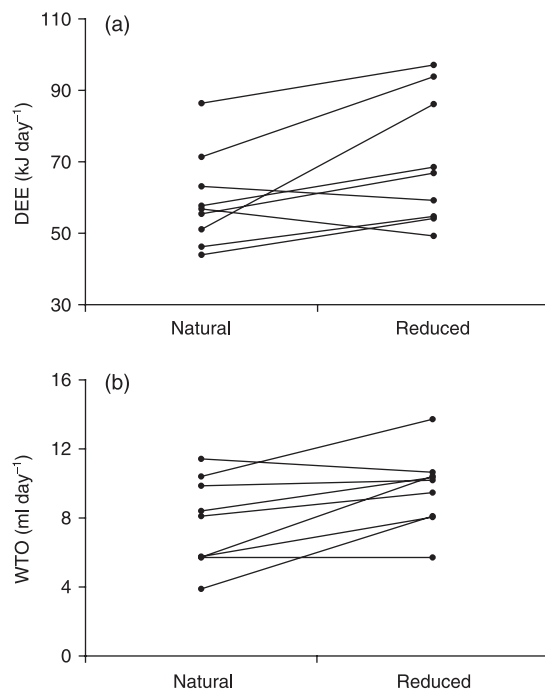


Fig. 2. (a) Daily energy expenditure (DEE, kJ day⁻¹), and (b) water turnover (WTO, ml day⁻¹) of free-living individuals from unmanipulated group sizes (natural) and of the same individuals 2 days later when group sizes have been reduced by one-half (reduced). Each line and filled circle denotes a single individual.

WATER TURNOVER (WTO) AND WATER
ECONOMY INDEX (WEI)

WTO averaged 7.70 ± 2.57 ml day⁻¹ and WEI averaged 8.32 ± 2.56 ml kJ⁻¹ in natural group sizes (Fig. 2b). There were no effects of body mass or group size on WTO ($F_{1,6} = 0.21$, $P = 0.662$ for body mass and $F_{1,6} = 0.10$, $P = 0.763$ for group size) or WEI ($F_{1,6} = 0.27$, $P = 0.625$ for body mass and $F_{1,6} = 0.01$, $P = 0.942$ for group size). However, WTO significantly increased and WEI significantly decreased when group sizes were reduced (two-sample $t = 3.05$, $P = 0.016$ for WTO and $t = 9.98$, $P < 0.001$ for WEI).

Discussion

Many laboratory studies have shown that small mammals in large groups have lower individual energy expenditures than those in small groups or single individuals (e.g. Contreras 1984; Hayes *et al.* 1992;

Putala, Hohtala & Hissa 1995; Ostner 2002; Kaufman *et al.* 2003). However, measurements of thermoregulatory savings due to group living in free-living small mammals are hard to determine and results have so far been inconclusive (Berteaux *et al.* 1996; Kenagy & Pearson 2000). In the current study we show that larger groups of African Four-Striped Grass Mice (*Rhabdomys pumilio*) expend less energy than smaller groups at temperatures below the thermoneutral zone in the laboratory. This effect was particularly pronounced at the lowest measured temperature (5 °C) in which oxygen consumption values were approximately 20% greater in groups of one to three animals than in groups of six to eight (Fig. 1). The largest difference in energy expenditure, as a result of differing group sizes, was one individual *vs* many (Fig. 1). Canals *et al.* (1989) found that energy savings were not much greater when group size became larger than about five; our results are consistent with this finding. In addition, we found that free-living DEE was greater in groups that had experimentally been reduced by one-half. This may indicate that increased group size is associated with thermoregulatory savings. There is the possibility that the measured increase in DEE could somehow have been a stress response to the manipulation. However, this is unlikely to be the case for two reasons. First, variation in group size is a natural phenomenon experienced in Four-Striped Grass Mice (mice sometimes leave their group and sleep in another nest, and later come back to their own group), and although group size is normally fairly stable, the number of individual animals in one group might vary naturally from one day to the next (C. Schradin, unpublished data). Second, even if our manipulations did stress the mice, Four-Striped Grass Mice respond to stressors in captivity by decreasing activity (N. Pillay, unpublished data), such that the effect of stress would be more likely to decrease the measured DEE than to increase it. Interestingly, we found no relationship between natural group size and DEE. DEE is dependent on many different biological factors that vary spatially between the groups, for example: the energy costs of foraging; the distance travelled daily (mice can travel 280–1600 m per day); interactions with conspecifics and other species; the differences in distribution of different food plants; the differences in food availability between territories and many more such spatially variable factors (Schradin 2006b). In field measurements, it is not readily possible to standardize and to correct for these factors. We suggest that the lack of a relationship between DEE and group size between mice from different groups is most probably due to these factors. Additionally, the lack of a group size effect may be a consequence of the small sample size ($n = 9$) used in the study as indicated by the separate analyses of covariates. The power of the original statistical test was $\beta = 0.57$ (effect size = 0.4) and an additional 64 samples would have been required to detect significant differences in DEE between mice

from naturally different group sizes; such large samples are not available at our field site.

We modelled the effects of experimentally reducing groups of free-living mice by one-half on the resultant DEE using the relationship between group size and DEE obtained in the laboratory (equation 3, this study). This showed that the expected increase in DEE for free-living mice would be 9.3 kJ day⁻¹. This compared well with the actual measured increase of 10.8 kJ day⁻¹. Thus, increases in overnight energy expenditure (due to thermoregulatory losses) may well account for almost all the difference in DEE between control and reduced group sizes of the free-living mice in our study. Moreover, we found that mice used more water less efficiently when they were in groups that were reduced. This is consistent with the idea that water turnover increases when metabolic rates are higher. Overall values of DEE and WTO were intermediate between allometric predictions for mesic and arid species: DEE values were 32% lower than predictions for mesic rodents but 47% greater than predictions for desert rodents (Nagy, Girard & Brown 1999); WTO values were 35% lower than predictions for non-desert eutherians but 51% greater than predictions for desert eutherians (Nagy & Peterson 1988). Although these mice may not inhabit purely arid environments, frugal use of water and energy is necessary when living in a desert habitat, such as the succulent karoo.

Unlike most desert habitats, winter rainfall patterns are predictable in the succulent karoo. This is one reason that the area is suggested to be able to support many succulents and perennials, providing a characteristic flush of new vegetation and flowers in the winter (Cowling *et al.* 1999) and a high density of rodents (Schradin 2006a). During the summer, food and water are scarce and Four-Striped Grass Mice lose around 12% of body weight from the beginning (December) through to the end (April) (Schradin & Pillay 2005b); mortality rates may reach 27%. Minimal measured values of ambient temperature are low (around 5 °C: M. Scantlebury, personal observation), so even during the warmest period of the year there may be substantial benefits to be gained from huddling. However, the energetic benefits of huddling might be expected to be especially important at the very beginning of winter, when minimal temperatures fall below freezing, but before any significant plant growth has taken place (Cowling *et al.* 1999). This is shown to be a stressful time for these mice as mortality rates may reach 70% during this period (Schradin & Pillay 2005b).

Group living may arise when animals inhabit adverse environments, or depend on sparse and widely dispersed food (Emlen 1997; Cockburn 1998). This may enable sufficient resources to be accrued from the environment (Alexander 1974; Macdonald 1980; Johnson, Macdonald & Dickman 2000) while energy costs are minimized (Ostner 2002; Rhind 2003). However, few studies have directly examined the importance of energy within group-living systems

(although see Anava *et al.* 2001; Scantlebury *et al.* 2002). In some circumstances, thermoregulatory savings due to huddling are suggested to be one possible mechanism driving the evolution of group living (Beauchamp 1999). Four-Striped Grass Mice are unusual in that they live in groups in a xeric habitat, whereas they are solitary in mesic grasslands (Schradin & Pillay 2003, 2004). One obvious difference between the two environments is that the variations in ambient temperature (both daily and annually) and food resources are much greater in the desert. Thus, mice stand to gain more energetic and water-conserving benefits from huddling in the desert than in the grasslands – which could be one of the possible reasons for the observed differences between the social structures between populations of Four-Striped Grass Mice in southern Africa.

In conclusion, we show for the first time that, in free-living mammals, DEE and WTO values are lower in natural group sizes than in group sizes that have been artificially reduced. Differences in DEE are consistent with measured energy savings in the laboratory of the same population of mice under controlled conditions. Observed differences in free-living DEE are therefore likely to be produced by differences in thermoregulation. Thermoregulatory savings are one possible reason that mice live in groups in deserts whereas they are solitary in grasslands.

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