Body mass as an estimate of female body condition in a hibernating small mammal

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Abstract
In hibernating squirrels, the amount of energy stored as fat may influence several important demographic traits, but is difficult to quantify in living animals. Thus, several non-destructive indices of body condition are used, including simple indices that use body mass and scaled indices that correct body mass for structural size. However, the accuracy of these indices for hibernating squirrels is poorly known. We used measurements of total body electrical conductivity (TOBEC) from adult female Golden-mantled Ground Squirrels (*Callospermophilus lateralis*) to characterize body composition (lean mass versus fat mass) and condition (fat stores) at multiple stages in the circannual cycle. Body mass explained a high proportion of the variation in fat mass during the emergence and pre-hibernation stages, but less during the reproduction stage. Contrary to expectation, correcting for structural size did not markedly improve the condition index. Our results suggest that body mass is a good estimate of body condition during the periods of emergence and pre-hibernation fattening, and therefore may be useful to predict important components of fitness such as reproductive success and overwinter survival.

Key words: Body mass; body condition; condition index; mass-length residuals; fat; ground squirrel; *Callospermophilus lateralis*

Introduction

Seasonal variation in energy supply is a central problem for many mammals, which may respond to periods of environmental energy shortage by storing energy, reducing energy expenditure, or both (Humphries *et al.* 2003). Hibernation, which reduces metabolic demands during winter, is one life-history adaptation to seasonal energy scarcity, but sufficient energy stores are essential to its success (Pulawa and Florant 2000).

In hibernating squirrels, the amount of energy stored as fat may influence several important demographic traits such as overwinter survival (Murie and Boag 1984; Lenihan and Van Vuren 1996), timing of reproductive maturity (Barnes 1984), male breeding effort (Delehanty and Boonstra 2011), female reproductive success (Dobson and Michener 1995; Rieger 1996), offspring sex allocation (Allainé *et al.* 2000), and natal dispersal (Nunes and Holekamp 1996; Neuhaus 2006). Additionally, estimating fat stores is essential for bioenergetic models of hibernation, which can be used to project distribution changes of hibernating species under changing climatic conditions (Humphries *et al.* 2002). However, quantifying body condition (defined here as fat stores, in grams; Kiell and Millar 1980; Dark *et al.* 1989) is difficult to do non-destructively. Because determining the effects of body condition on future life-history outcomes requires that the animal survive measurement, several non-destructive indices for estimating condition have been developed (Schulte-Hostedde *et al.* 2005; Peig and Green 2010). These include simple condition indices that use body mass (e.g., Hock 1960), and scaled condition indices that attempt to correct body mass for structural size (e.g., Reid 1988). Many studies use total body mass as a simple condition index, with the implicit assumption that greater mass reflects greater relative fat stores (Barnes 1984; Sauer and Slade 1987; Lenihan and Van Vuren 1996; Neuhaus 2003; Lane *et al.* 2011). However, larger animals may have greater mass due to larger structural size (skeleton and associated lean tissue) instead of greater fat stores (Dobson 1992). Thus, some studies use a scaled condition index based on residuals derived from a regression of body mass on structural size, with the expectation that correcting body mass by the structural size of an individual improves the estimate of its condition (Bachman 1993; Dobson

and Michener 1995; Dobson *et al.* 1999; Allainé *et al.* 2000). Positive residuals suggest the animal contains more tissue (presumably fat) than predicted for a given structural size, while negative residuals suggest the animal contains less tissue than predicted for a given structural size.

Scaled indices are appealing because they correct for variance in body mass that is unrelated to energy stores, but available evidence indicates that size-corrected measures do not necessarily improve estimates of body condition compared to use of body mass alone (Krebs and Singleton 1993; Green 2001; Schamber *et al.* 2009). However, most evaluations of condition indices have focussed on mammals that do not store fat for hibernation or energy reserves, and the poor relationship between the scaled condition index and measured fat content may occur because residuals of these relatively lean species primarily reflect differences in protein or water content rather than fat (Schulte-Hostedde *et al.* 2001). Scaled indices might be more appropriate for species in which fat content is a greater proportion of body mass, such as hibernators (Schulte-Hostedde *et al.* 2001), but the predictive ability of simple versus scaled condition indices for hibernating squirrels is poorly known.

Fat storage in hibernating squirrels follows circannual cycles of accumulation and depletion (Buck and Barnes 1999), reflecting seasonal changes in the balance between energy acquisition and expenditure (Kenagy *et al.* 1989). For an index to be an appropriate estimate of body condition, it should explain a high proportion of the variation in fat storage, preferably across multiple stages of the circannual cycle. Adult females are often excluded from condition index validation because of the confounding effect of fetal lean tissue elaboration during gestation (Krebs and Singleton 1993; Schulte-Hostedde *et al.* 2005), yet energetic costs associated with hibernation and reproduction deplete fat stores, and therefore affect body condition, in adult females as well as males (Kenagy 1989; Michener and Locklear 1990; Buck and Barnes 1999). In this paper we use measurements from adult female Golden-mantled Ground Squirrels (*Callospermophilus lateralis*) to evaluate fat stores during four major stages (emergence, reproduction, post-reproduction, and pre-hibernation) in their circannual cycle. Our goal is to assess body mass as a simple index of body condition in each stage, and determine if using a scaled index improves estimates of body condition.

Methods

We studied Golden-mantled Ground Squirrels over three years (2003–2005) in the northern Sierra Nevada mountains of California. These squirrels are locally abundant, medium-sized (200–300 g), and relatively well-known both ecologically and physiologically (Bartels and Thompson 1993).

Our study was conducted in the Plumas National Forest (40.004012°N, 120.810829°W) near Quincy, California, at an elevation of ~2100 m. In this area, adults emerge from hibernation in May and pups are weaned in late July; all squirrels gain weight during September before immerging into hibernation in October. Gestation in Golden-mantled Ground Squirrels is 28 days (Cameron 1967) and weaning occurs when pups reach 30 days old (Phillips 1981). We divided the active season into four circannual stages, defined broadly to encompass individual variation in circannual timing: emergence, 15 May–15 June (emergence from hibernation through mating and early gestation); reproduction, 16 June–31 July (late gestation through lactation); post-reproduction, 1 August–31 August (after lactation but before late summer fattening becomes pronounced); and pre-hibernation, 1 September–early October (when pre-hibernation fattening occurs). Because we did not determine reproductive status for all females in this study, our sample may have included non-reproductive females.

We captured adult female squirrels with Tomahawk live traps (Model 201, Tomahawk Live Trap Co., Hazelhurst, Wisconsin, USA) baited with rolled oats and black oil sunflower seeds coated with peanut butter. Traps were set in the early morning and checked mid-morning. Our methods were conducted according to a protocol approved by the Animal Care and Use Committee of the University of California, Davis, and followed guidelines approved by the American Society of Mammalogists (Sikes *et al.* 2016). At first capture, squirrels were fitted with a uniquely numbered metal tag (Self-piercing fish tag, Style 1005-1, National Band & Tag Company, Newport, Kentucky, USA) in each ear for permanent identification. We attempted to capture all squirrels monthly, but due to differential trapping success not all squirrels were captured each month. We transported captured squirrels to a laboratory near Quincy, where we anesthetized them with an intramuscular injection of ketamine hydrochloride (100 mg/ml). We recorded body mass to the nearest 0.1 g using a portable electronic balance and body length (measured as tip of nose to anus) to the nearest 0.1 cm (Pulawa and Florant 2000). We used body length as a measure of structural size (Bachman 1993; Allainé *et al.* 2000); our measurements of body length showed good repeatability for individuals recaptured in the same stage (Pearson correlation $r = 0.83$, $n = 5$). We quantified body fat using an EM-SCAN SA-3000 body composition analyzer (EM-SCAN, Springfield,

Illinois, USA; no longer available from the manufacturer) to measure total body electrical conductivity. Total body electrical conductivity (TOBEC) is a nondestructive method to analyze the body composition of animals (Scott *et al.* 2001) that has been used to obtain estimates of lean and fat mass from free-living small mammals (Walsberg 1988; Koteja 1996), including ground squirrels (Nunes and Holekamp 1996; Buck and Barnes 1999; Pulawa and Florant 2000). The TOBEC method uses electrical current, which travels differentially through fat versus lean tissue, to generate measures of electrical resistance; resistance measures are then converted to fat mass using species-specific calibration equations (Bachman 1994; Koteja 1996; Walsberg 1998; Scott *et al.* 2001).

EM-SCAN readings are known to vary with animal movement during measurement, differences in gut contents, changes in ambient temperature, and changes in body temperature greater than 4°C (Walsberg 1988; Scott *et al.* 2001). To minimize variation due to movement, we placed immobilized squirrels on a plastic sample tray and lightly restrained them with rubber bands to maintain each squirrel in the same position (dorsoventrally, ventral side down, with the tail tucked under the body). To minimize variation due to gut contents, we only trapped squirrels early in the morning (as foraging began) and did not provide food or water until after TOBEC measurement. To minimize variation due to ambient temperature, we performed measurements in a laboratory at a field station. Anesthesia often causes a drop in body temperature; throughout our study, however, the mean change in body temperature was -1.6 ± 0.3°C (SE), and no individuals lowered their body temperatures more than 4°C. Body composition was calculated as the mean of five replicate measurements; we recorded seven replicate measurements and then discarded the highest and lowest values, though variation in measurements was minimal (coefficient of variation = 0.03). We determined lean mass (M_L) using the calibration curve for Golden-mantled Ground Squirrels:

$$M_L = 18.0 + 0.3M_B + 1.2\sqrt{L_B \times EM}$$

where M_B is body mass, L_B is body length, and EM is the EM Scan measurement ($r^2 = 0.98$; Pulawa and Florant 2000). We calculated fat mass by subtracting lean mass from body mass.

We characterized the body composition (lean mass versus fat mass) and condition (fat mass) of adult female ground squirrels during emergence, reproduction, post-reproduction, and pre-hibernation stages. Because female energetic needs shift throughout the active season from expenditure on reproduction to acquisition before hibernation (Kenagy *et al.* 1989),

potentially changing the relationship between body mass and fat mass, we considered each circannual stage separately. We assessed fat stores of 23 adult female Golden-mantled Ground Squirrels; seven were measured in a single circannual stage, six were measured in two circannual stages, eight were measured in three circannual stages, and two were measured in all four stages. Sample size varied by stage, and each female was included only once per stage. If females were measured more than once within the same stage, we randomly selected a single measurement from those taken in the same year ($n = 5$ females), and we considered measures to be independent if taken in different years ($n = 2$ females; Broussard *et al.* 2005). We also tried averaging measurements for the same female within a year, but the results were similar whether we averaged or chose measurements at random. We used analysis of variance (ANOVA) with Tukey's HSD *post-hoc* tests to test for significant differences in mean body length and mean body composition among circannual stages. We used linear regression to examine the relationship between body length and mass by each circannual stage.

Next, we used bivariate linear regression to evaluate the relationship between body mass and fat mass for each circannual stage, and also the relationship between mass-length residuals, calculated from regressing body mass on body length, and fat mass. In addition, because percent fat (fat mass/total body mass) is sometimes used as a measure of body condition in hibernating squirrels (Barnes 1984; Nunes and Holekamp 1996; Neuhaus 2003) we performed the same regressions for percent fat as we did for fat mass. The use of body mass as a variable in both the TOBEC calibration equation and as a predictor of fat mass may introduce some underlying structure to the data, with the potential to inflate the r^2 values. While this is unavoidable, we therefore report r^2 values associated with linear regressions for comparison among stages and indexes, and without associated significance tests (Wasserstein and Lazar 2016).

Finally, because our data contained substantial individual and annual variation in percent fat, which may confound relationships between condition indices and percent fat inferred through linear regression, we fitted linear mixed models with individual female identity and year as random effects, and circannual stage and condition index specified as fixed effects. Models were estimated with Bayesian inference. We used a Bayesian, mixed-effects approach for two reasons: 1) the hierarchical structure of our data suggested the use of mixed effects models that produce more accurate estimates of all parameters, and 2) Bayesian approaches more accurately partition variance among mixed effect parameters than

likelihood-only approaches (McElreath 2016). We developed four models: two with fat mass (in grams) as the response variable, predicted by either mass or mass-length residuals, and two with percent fat as the response variable, predicted by either mass or mass-length residuals. We included all measurements ($n = 61$) of the 23 adult females in this analysis.

We used a model comparison approach to evaluate the ability of each index to predict fat mass and percent fat. Specifically, we used the Watanabe-Akaike Information Criterion (WAIC) to rank models, based on WAIC differences (ΔWAIC) and Akaike weights. Such values are analogous to other information criteria, where low ΔWAIC values indicate preferred model, and high weight indicates increased probability that the model will successfully predict new data (Gelman *et al*. 2014; McElreath 2016). All analyses were run in R version 3.5.2 (R Development Core Team 2016); we used the packages RStan (Stan Development Team 2016) and rethinking (McElreath 2016) to fit and compare mixed models, and ggplot2 (Wickham and Chang 2013) to plot figures.

Results

Lean mass of adult female Golden-mantled Ground Squirrels varied among circannual stages ($F_{3,51} = 3.52$, $P = 0.02$; Table 1), and was lowest at emergence from hibernation and highest before immergence. Estimated fat mass also varied among circannual stages ($F_{3,51} = 7.35$, $P < 0.001$), and was lowest at emergence from hibernation and highest before immergence. Percent fat varied among circannual stages ($F_{3,51} = 5.90$, $P = 0.002$), and appeared stable throughout the first three stages before showing a sharp increase in the pre-hibernation stage. Additionally, mixed models revealed a generally positive effect of the pre-hibernation stage on fat mass, after controlling for year, individual, and mass or mass-length residual (Table 2).

The relationship between body mass and body length was positive during emergence ($r^2 = 0.55$, $n = 12$, $P < 0.01$), reproduction ($r^2 = 0.41$, $n = 15$, $P < 0.01$), and post-reproduction stages ($r^2 = 0.33$, $n = 16$, $P = 0.02$), but was no longer apparent during the pre-hibernation stage ($r^2 = 0.00$, $n = 12$, $P = 0.98$; Figure 1).

Body mass explained a very high proportion of the variation (93–96%) in fat mass during the emergence, post-reproduction, and pre-hibernation stages, but a lower proportion (84%) during the reproduction stage (Figure 2). Correcting for structural size, as measured by head and body length, did not improve fit within any stage: the proportion of variation explained by mass-length residuals was less than that for the simple index based on body mass during the emergence, reproduction, and post-reproduction stages (57–70%), and equivalent to that explained by body mass during the pre-hibernation stage (96%).

Overall, a similar pattern was evident for the analysis based on percent fat. Body mass explained a moderate to high proportion of the variation in percent fat during the emergence ($r^2 = 0.79$), post-reproduction ($r^2 = 0.69$), and pre-hibernation stages ($r^2 = 0.91$), but a lower proportion during the reproduction stage ($r^2 = 0.56$). Correcting for structural size did not markedly improve fit within most stages, though mass-length residuals did explain a significant proportion of the variation in percent fat (emergence $r^2 = 0.61$, post-reproduction $r^2 = 0.46$, pre-hibernation $r^2 = 0.91$). Correcting body mass by body length improved model fit only in the reproduction stage ($r^2 = 0.86$). While both mass and mass-length residuals showed strong positive effects on fat mass and percent fat, WAIC metrics showed a clear preference for the mass models ($w_i = 1$, ΔWAIC=0.0; ΔWAIC for the second model >69 for fat grams and >20 for percent fat; Table 2).

Discussion

Our results suggest that body mass is a useful estimate of body condition during the critical periods of emergence from hibernation and pre-hibernation fattening, and perhaps during the post-reproductive period, supporting the use of body mass as a simple index to predict important components of fitness such as female reproductive success (Rieger 1996) and overwinter survival (Murie and Boag 1984). Body mass

TABLE 1. Mean length and body composition ($\pm$ 1 SE) of adult female Golden-mantled Ground squirrels (*Callospermophilus lateralis*) near Quincy, California, from 2003 to 2005, by circannual stage.

	Emergence 15 May–15 June	Reproduction 16 June–31 July	Post-reproduction 1–31 August	Pre-hibernation 1 September–1 October
n	12	15	16	12
Mean length (cm)	17 ± 0.4	18 ± 0.3	17 ± 0.3	18 ± 0.3
Mean total mass (g)	158 ± 8.5[a1]	175 ± 5.3	167 ± 5.9[a1]	198 ± 9.4[b1]
Mean lean mass (g)	124 ± 4.8[a2]	135 ± 3.4	130 ± 3.6	142 ± 4.5[b2]
Mean fat mass (g)	35 ± 3.9[a3]	39 ± 2.1[a3]	38 ± 2.5[a3]	56 ± 5.1[b3]
Mean percent fat	21 ± 1.7[a4]	22 ± 0.8[a4]	22 ± 0.8[a4]	28 ± 1.4[b4]

[a#]Statistically different value(s) from [b#] across circannual stages for that variable, according to Tukey HSD *post-hoc* test.

TABLE 2. Parameter estimates from linear mixed models predicting fat mass (left of dividing line) and percent fat (right of dividing line) of adult female Golden-mantled Ground Squirrels (*Callospermophilus lateralis*), with mass and mass-length residuals used as predictor variables. Estimates in bold indicate fixed effects that are reliably positive. Fixed effects of circannual stage (reproduction, post-reproduction, and pre-hibernation) are compared to the baseline of the emergence stage. Female ID and Year were fitted as random effects with varying intercepts. ΔWAIC values and weights (w_i) are compared between the two models with the same outcome variable.

	Fat grams~mass			Fat grams~MLResiduals			Percent Fat~mass			Percent Fat~MLResiduals		
	Mean ± SD	Lower 0.89	Upper 0.89	Mean ± SD	Lower 0.89	Upper 0.89	Mean ± SD	Lower 0.89	Upper 0.89	Mean ± SD	Lower 0.89	Upper 0.89
Intercept	−35.79 ± 3.36	−40.92	−30.36	40.07 ± 2.70	36.11	43.85	0.77 ± 2.33	−2.68	4.15	22.86 ± 1.65	20.43	25.43
Mass	**0.45 ± 0.02**	**0.42**	**0.48**	—	—	—	**0.13 ± 0.01**	**0.11**	**0.15**	—	—	—
Mass-Length Residual	—	—	—	**0.46 ± 0.04**	**0.40**	**0.52**	—	—	—	**0.14 ± 0.01**	**0.12**	**0.16**
Reproduction	−1.86 ± 1.19	−3.72	0.03	1.36 ± 1.99	−1.86	4.38	−0.25 ± 0.66	−1.29	0.82	0.62 ± 0.77	−0.59	1.85
Post-Reproduction	−1.42 ± 1.30	−3.50	0.63	0.03 ± 2.25	−3.56	3.49	−0.49 ± 0.77	−1.75	0.69	−0.08 ± 0.94	−1.54	1.42
Pre-Hibernation	2.23 ± 1.78	−0.43	5.15	**6.45 ± 2.95**	**2.08**	**11.35**	0.22 ± 1.12	−1.46	2.09	1.29 ± 1.30	−0.71	3.45
ΔWAIC	0.0			69.4			0.0			20.7		
w_i	1			0			1			0		

was less reliable during the reproductive stage, when females likely varied in their reproductive status. Because variation in reproductive status is associated with differences in body composition (Holekamp and Nunes 1989), combining non-reproductive, pregnant, and lactating females likely weakened the relationship between body mass and fat mass; this pattern was more pronounced when condition was defined as percent fat. In pregnant females, increased mass reflects additional lean fetal tissue instead of fat mass (Boswell *et al.* 1994). Reproductive females also vary in litter size (McKeever 1964), and hence fetal mass.

Our study faced two limitations, besides small sample sizes. First, we did not directly measure fat content of squirrels through destructive sampling and chemical extraction. Second, although the TOBEC method has been validated for Golden-mantled Ground Squirrels (Pulawa and Florant 2000), we did not calibrate the TOBEC machine for our population of the species. TOBEC equations derived from one population have been successfully applied to new individuals in another rodent species (Dickinson *et al.* 2001), but the accuracy of TOBEC among populations of ground squirrels is unknown. Consequently, our measurements of fat content are estimates only.

Adult female Golden-mantled Ground Squirrels showed seasonal changes in body composition consistent with other hibernating squirrels, gaining both lean mass and fat mass during the active season (Kiell and Millar 1980; Rickart 1982). Other studies have demonstrated that ground squirrels appear to catabolize both lean and fat tissues to fuel hibernation, but restore these once the vegetative growing season begins (Jameson and Mead 1964; Kiell and Millar 1980; Pulawa and Florant 2000). A late season gain in fat mass is characteristic of hibernating species (Kunz *et al.* 1998; Buck and Barnes 1999; Hilderbrand *et al.* 2000), and it was during this stage that fat mass was best predicted by body mass.

In general, our finding that body mass explained substantial variation (84–96%) in female fat mass is consistent with those of previous studies that combined male and female squirrels: body mass explained 76% of the variation in fat mass in Belding's Ground Squirrels (*Urocitellus beldingi*) collected throughout the active season (Morton and Tung 1971), and 82% of the variation in percent fat in Arctic Ground Squirrels (*Urocitellus parryii*) held in captivity (Lee *et al.* 2011).

Surprisingly, adjusting for structural size using body length did not improve estimates of body condition, except during the reproduction stage for estimates of percent fat. Body length and body mass were linearly related in our squirrels at emergence, supporting the premise that greater mass in some stages was due, at least in part, to larger structural

size. However, the relationship between body length and mass almost disappeared by the pre-hibernation stage (Figure 1). Because the regression of body mass on length had a slope of zero in the pre-hibernation stage, and the magnitude of residuals was equal to relative body mass, the fit of mass-length residuals was identical to that of body mass in this stage.

We suggest three reasons why the scaled index may have performed poorly. First, measures of structural size may be particularly susceptible to measurement error (Yezerinac *et al*. 1992; Blackwell *et*

al. 2006; Martin *et al*. 2013). Although we reduced this error by measuring body length on anesthetized squirrels, which are more amenable to measurement than active, unanesthetized squirrels, and this measurement displayed high repeatability within stage, some measurement error remained. Second, measures of structural size such as body length may sometimes be a poor indicator of lean mass that is not associated with energy storage. As with the relationship between body length and total mass noted above, the strength of the relationship between body length
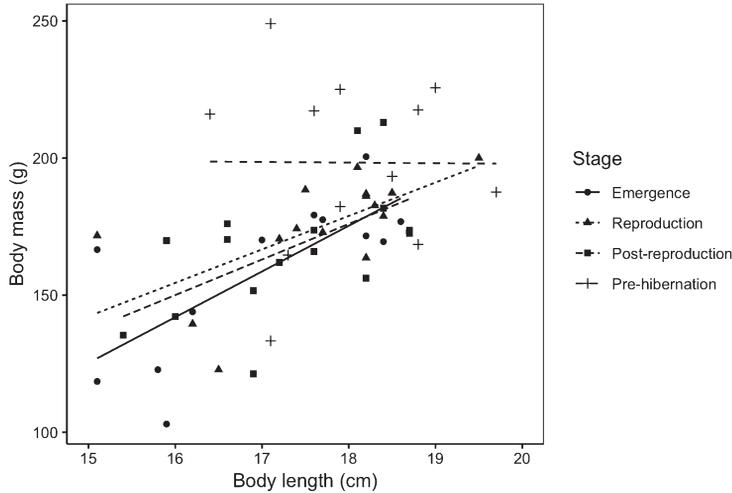
Figure 1. Linear relationships between adult female Golden-mantled Ground Squirrel (*Callospermophilus lateralis*) body mass and body length, by circannual stage.
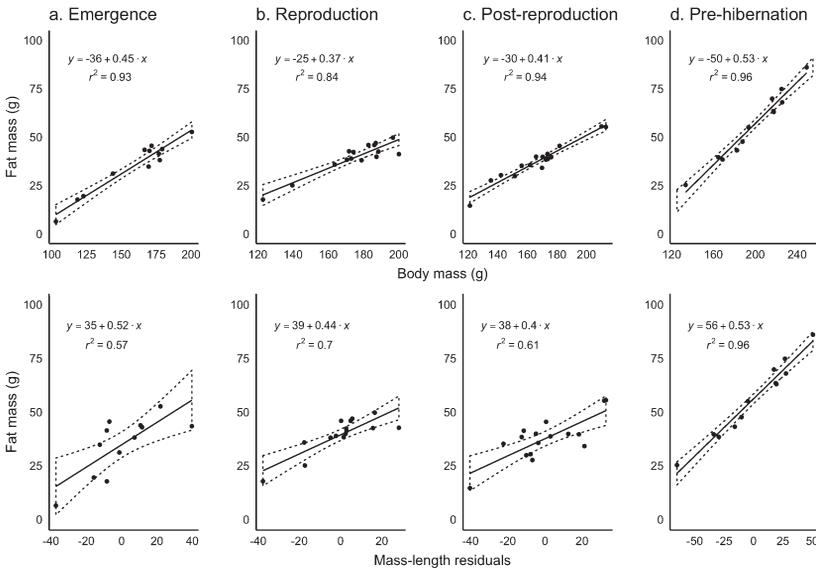
Figure 2. Linear relationships between adult female Golden-mantled Ground Squirrel (*Callospermophilus lateralis*) total body mass and fat mass (top row), and mass-length residuals and fat mass (bottom row), by circannual stage (columns). Dotted lines represent a 95% CI (two standard errors).

and lean mass declined over the active season (from $r^2 = 0.64$ at emergence to $r^2 = 0.01$ before hibernation). Some individuals that emerged from hibernation with lower body mass than expected for their body size still had substantial fat stores; perhaps these individuals preferentially lost lean mass during hibernation (Pulawa and Florant 2000) to retain fat stores necessary for initiating reproduction. Finally, although body length commonly has been used in ground squirrel studies (Morton *et al.* 1974; Kiell and Millar 1980; Pulawa and Florant 2000), it may simply be a poor measure of structural size. Other studies have used breadth across the zygomatic arches as a measure of structural size (Dobson *et al.* 1999; Viblanc *et al.* 2010). Zygomatic arch breadth has a significant but not especially strong linear relationship with body length for adult females of this species ($r^2 = 0.44$, $P < 0.01$, $n = 18$; C.P.W. unpubl. data), however, highlighting the uncertainty of using a single measure to quantify as complex a trait as structural size.

Body condition is an important trait in the life history of ground squirrels, but measuring condition directly requires sacrificing the animal. Our results suggest that the simple measure of body mass is a useful indicator of body condition, especially early and late in the active season, and that scaled indices do not improve on mass estimates during most stages in the circannual cycle.

Acknowledgements

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