

**HIBERNATION BIOLOGY OF RICHARDSON'S GROUND SQUIRRELS:
HIBERNACULUM SYSTEMS AND ENERGY UTILIZATION**

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DEDICATION

To my first son, Nathan, who has been with me through it all.

Daddy's gonna stay home tonight, and no, tiger,

I don't have to work on my thesis.

Wanna wrestle?

And to my second son, Mackenzie, who has never known

this task but has been nonetheless affected.

Daddy's gonna try to stay home most nights, and no, slugger,

I won't have to work on my thesis.

Wanna wrestle when ya grow up a little?

ABSTRACT

I studied free-living Richardson's ground squirrels (*Spermophilus richardsonii*) using telemetry and total body electrical conductivity (TOBEC) to evaluate overwinter energy utilization and the impact of seed caching on body composition of males. I excavated 51 hibernation systems and found that 66% of 35 males cached 1 to 4 species of seed in the hibernaculum. Pre-emergent euthermicity was shorter for 3 non-caching (0.7 ± 0.2 days) than for 13 caching males (4.0 ± 2.8 days), and metabolic predictions of overwinter mass loss approximated actual loss for non-caching males, but over-estimated mass loss for caching males. I concluded that caching males recouped some of the mass lost during hibernation by eating the cache during the longer period of pre-emergence euthermicity. Based on TOBEC, the recouped mass included both fat and lean tissue. I suggest that caching in one year is a cost of reproduction that offsets the energetic demands of mating the following year.

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LIST OF ABBREVIATIONS

FBM	Fat body mass
LBM	Lean body mass
LRC	Lethbridge Research Centre
pFBM	Proportional loss of fat body mass
pLBM	Proportional loss of lean body mass
T _a	Ambient temperature
T _b	Body temperature
TOBEC	Total body electrical conductivity
T _{soil}	Soil temperature
V _b	Hibernaculum volume
VO ₂	Volume of oxygen consumed
V _s	Hibernation system volume

Chapter I

INTRODUCTION

Richardson's ground squirrels (*Spermophilus richardsonii*) in southern Alberta, Canada, hibernate for 3-8 months depending on the age and sex of the squirrel (Michener 1979, 1983, 1985, 1992, 1993, 1998; Michener & Locklear 1990a). Hibernation is a strategy that is generally accepted as a method of energy conservation during periods of poor food availability and/or harsh climatic conditions. The strategy is dependent on reducing metabolic energy expenses during hibernation below what would be required to remain active. Mature mammals are able to maintain a relatively constant core body temperature (homeothermy) during normal activity regardless of ambient temperature by producing heat metabolically (endothermy), an energetically expensive process. Small (<5 kg) mammalian hibernators reduce their metabolic rates by becoming heterothermic, allowing body temperature to more or less reflect that of the ambient temperature (e.g., Barnes 1989; Scheck and Flaherty 1976; Michener 1992; Wang 1978, 1979). Torpor, which describes the lethargic condition of animals during these bouts of cooler body temperatures, is frequently interrupted in all deep hibernators by periodic arousals to normothermic temperatures (Michener 1992; Scheck and Fleherty 1980; Wang 1973, 1978, 1979). These brief inter-torpor arousals account for <10% of the time spent in hibernation by Richardson's ground squirrels, yet amount to 70% of the total energy expended during hibernation (Wang, 1978). However, by maintaining heterothermy for >90% of the time in hibernation, Wang (1978) estimated that the overall energy savings during hibernation were 88% of the cost of remaining homeothermic for the same

duration.

The energetic demands of hibernation must be met by increasing the energy stored either in body tissues (endogenous), in the nest chamber (hibernaculum) in the form of food (exogenous), or by some combination of the two. Fat is favoured as the endogenous energy substrate for hibernation because of its higher energy density on a mass basis than either protein or carbohydrate (Mathews and van Holde 1996); animals can minimize the amount of mass they are required to gain to subsidize their hibernation energy demands if they gain fat instead of lean mass. Exogenous energy stores in the form of food caches in the hibernaculum have been reported for males, but not females, for Columbian ground squirrels (*S. columbianus*, Shaw 1926) and *S. richardsonii* (Michener 1992, 1993) and inferred for male but not female arctic ground squirrels (*S. parryii*, Buck and Barnes 1999a, 1999b; McLean and Towns 1981). The sexual difference in caching behaviour has been explained as the means by which males can reduce the amount of mass lost during hibernation and advance the state of testicular recrudescence, with the aim of improving their mating success during the mating season, which occurs almost immediately upon emerging from hibernation in the late winter (Barnes *et al.* 1986, 1987; Michener 1992, 1993; Michener and Locklear 1990a, 1990b).

I conducted this study, which is presented in three parts, over the 1997-1998 and 1998-1999 hibernation seasons, to assess the impact of seed caching behaviour on mass loss and body composition of male Richardson's ground squirrels. To assess body composition and thus measure the endogenous (fat) energy stores of animals, I used total body electrical conductivity (TOBEC). In the first part of this study, I address the

requirement for a species-specific calibration for TOBEC. I also evaluated the influence of several factors that have been reported to reduce the accuracy of TOBEC. In the second part of the study, I assessed the amounts of energy used by animals and changes in body composition for the entire hibernation season. Wang (1978, 1979) established that metabolic rates of Richardson's ground squirrels during hibernation are influenced by body temperature, ambient (soil) temperature in the hibernaculum, and the amount of time spent in the 4 periods of a torpor bout: arousal, euthermia, cooling, and torpor. I used TOBEC to determine the body composition of animals before they immersed into hibernation and again after they emerged in the spring, and then calculated the proportional hibernation losses of fat and lean tissue. During hibernation, I employed telemetric methods to measure the body temperature of hibernating ground squirrels. Body temperature was used to calculate the total amount of time spent in each period of a torpor bout over the hibernation season, and I used these times, soil temperatures, and data on metabolic rates (Wang 1978, 1979) to estimate oxygen consumption (VO_2) during hibernation. The proportional losses of fat and lean tissue were combined with the estimates of VO_2 to derive a prediction of mass loss based on energy utilization. I reasoned that this energy-based prediction of mass loss should be accurate for animals that relied solely on endogenous energy reserves, but would over-predict the amount of mass loss for animals that had exogenous (seed cache) as well as endogenous reserves. I anticipated that this over-prediction would be a means by which I could assess the impact of seed caching on body mass and composition.

In the third part of the study, I excavated hibernacula to assess which animals had

cached seed and to document the size and composition of the caches. I also extended the work of excavation to examine the system of tunnels that were connected to the hibernaculum. I excavated each hibernation system in its entirety, measured the depth, length, and diameter of all tunnels and chambers, and calculated tunnel and system volumes. I described the architecture of the hibernation systems of Richardson's ground squirrels by identifying patterns of tunnel and chamber design that helped generalize such a description.

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Chapter II

ESTIMATION OF LEAN BODY MASS OF RICHARDSON'S GROUND SQUIRRELS (*SPERMOPHILUS RICHARDSONII*) USING TOTAL BODY ELECTRICAL CONDUCTIVITY (TOBEC)

ABSTRACT

The use and value of total body electrical conductivity (TOBEC) as a means of estimating body composition are reportedly influenced by factors such as the presence of eartags, body temperature, and degrees of gut fullness and hydration. Species-specific calibrations that use multiple regression to include measures of body size are purported to improve the accuracy of TOBEC. I evaluated the influence of these factors on the estimation of lean body mass (LBM) using TOBEC for Richardson's ground squirrels (*Spermophilus richardsonii*). The presence of eartags caused an increase in TOBEC readings by 9.6 units ($t_{19} = 1.89, P = 0.037$) and thus inflated estimates of LBM by 2.5 g. TOBEC declined with a decrease in body temperature, which was corrected with the equation: Adjusted TOBEC = TOBEC + 185.4 - 4.4 * body temperature. Compared with fasting, re-fed animals gained 27.7 ± 1.9 g, which resulted in an increase in TOBEC of 83 units ($t_{13} = 9.46, P < 0.0001$). In turn, this resulted in significant increases in absolute estimated values of both LBM and fat body mass (FBM), and changes in the percentages of LBM and FBM. Dehydrated animals lost 27.6 ± 4.4 g when compared to their pre-dehydration mass. This loss of mass resulted in a decrease in TOBEC of 104 units ($t_{15} = 6.21, P < 0.0001$). All of the change in mass due to rehydration was partitioned by TOBEC into increased LBM, with no significant change in FBM. I found that the length of the forelimb was the co-factor that most improved the estimate of LBM using TOBEC

in the equation: $LBM = 0.25 * TOBEC + 8.16 * Forelimb - 174.0$ ($r^2 = 0.92$). The influence of these factors on the accuracy of TOBEC must be assessed to ensure that studies using TOBEC provide results based only on changes in animal body composition.

INTRODUCTION

On a short-term basis (hours, days or perhaps weeks), the energy costs of many animal behaviours are underwritten by feeding, whereas overnight or overday fasting and more prolonged periods without eating, such as during migration or hibernation, require animals to derive energy from body tissues. Fat yields more than twice as much energy per gram as either protein or carbohydrate and is the tissue that forms the principle substrate for energy metabolism during fasts. In southern Alberta, Canada, adult Richardson's ground squirrels (*Spermophilus richardsonii*) hibernate underground for up to 9 months (e.g. Chapter III, Michener 1979, 1983, 1992). Michener (1992, 1993) and I (Chapter III) reported that, as with some other species of ground squirrels, male but not female Richardson's ground squirrels cache seed in the hibernaculum. Thus, the strategy of using fat stores accumulated before hibernation is the only energy available to females, whereas fat may be augmented for those males with access to a seed cache. Energy utilization during hibernation of Richardson's ground squirrels prompted my interest in evaluating the fat stores of animals before and after hibernation.

Methods of evaluating body composition in animals include the calculation of ratio indices of condition that relate body mass and some measure of structural size, underwater weighing (densitometry), measuring uptake of fat-soluble gases, assessing the

dilution of hydrogen isotopes in body water, stable isotope analysis, and estimating body fat by measuring the thickness of the subcutaneous fat layer using calipers or ultrasound. Problems with these methods include the need for subject cooperation and/or expensive equipment, a high degree of invasiveness, and/or lingering questions about the accuracy of the estimations. Classically, the simplest and most reliable evaluation of how much fat an animal had stored requires chemical extraction of aliquot samples of the carcass with non-polar solvents such as ether or chloroform (Folch *et al.* 1957, Sawicka-Kapusta 1975, Dobush *et al.* 1985). However, this approach involves killing the animal and thus is undesirable in general and is particularly inappropriate for longitudinal studies or those species that are endangered. In 1982, Harrison and Van Itallie described a technology which has since become known as total body electrical conductivity (TOBEC). TOBEC is based on the observation that the electrical conductivity of fat is only 4 - 5% that of the lean tissues of the body (Harrison and Van Itallie 1982). Thus, variations in the conductivity of the body are primarily due to variations in the amount of lean tissue in that body. If the body is placed in an electric field generated by a solenoidal coil, a current will be induced within the body that is proportional to the amount of lean tissue present. That current in turn causes a change in the impedance of the coil, which can be measured and related to the amount of lean tissue in the body. Determination of the amount of fat in the body (fat body mass or FBM) requires the calibration of the device for known lean body masses (LBM), usually by chemical carcass extraction, and then subtracting the LBM from the total body mass to derive the FBM. The body in the electric field must be stationary, with little or no muscle activity, and so requires that

animals are anaesthetized. Although death of a sample of animals is required to derive the calibration for each new species studied (Asch and Roby 1995), once this calibration is done, no other animals need be killed, and longitudinal as well as cross-sectional studies are made possible. TOBEC has been used to estimate body composition in a variety of mammals and birds, including humans (e.g. Harrison and Van Itallie 1982, Van Loan *et al.* 1987, Van Loan and Mayclin 1987), rats (*Rattus norvegicus*, Bracco *et al.* 1983, Cunningham *et al.* 1986), pigs (species not given, Fiorotto *et al.* 1987, Keim *et al.* 1988), Belding's ground squirrels (*Spermophilus beldingi*, Bachman 1994, Nunes and Holekamp 1996), arctic ground squirrels (*S. parryii*, Buck and Barnes 1999), prairie voles (*Microtus ochrogaster*, Voltura and Wunder 1998), northern bobwhites (*Colinus virginianus*, Roby 1991; Frawley *et al.* 1999), American woodcocks (*Philohela minor*, Morton *et al.* 1991), Gambel's quail (*Callipepla gambelii*, Walsberg 1988), and northern cardinals (*Cardinalis cardinalis*, Burger 1997).

Several factors may affect the TOBEC reading and thus the accuracy of estimates of LBM (and FBM). Despite early attempts to derive calibration equations that could be used with a variety of related or similar species (e.g. Castro *et al.* 1990; Walsberg 1988), species-specific calibrations are required. Unangst and Wunder (2001) demonstrated that different TOBEC calibration equations were required for meadow voles (*Microtus pennsylvanicus*) and prairie voles (*M. ochrogaster*), even though the size and morphology of these 2 species was very similar. Calibration should be done using animals with a range of body conditions that reflect the range in the population to be studied (Burger 1997; Frawley *et al.* 1999; Unangst and Wunder 2001).

The position of the animal's body within the coil (Walsberg 1988, Roby 1991, Bachman 1994) and variations in body shape (Harrison and Van Itallie 1982, Klish *et al.* 1984, Fiorotto *et al.* 1987) are important factors influencing TOBEC reading. Roby (1991) found that testing bobwhites with and without the head constrained in a net changed the TOBEC reading significantly. Parameters designed to reflect differences in body shape (girth/length, girth/length², weight/length, and weight/length²) all improved the prediction of LBM from TOBEC in pigs and rabbits (Fiorotto *et al.* 1987). The accuracy of estimates of body composition in voles was improved when body mass was included in the calibration equation and a multiple regression approach was used (Voltura and Wunder 1998, Unangst and Wunder 2001).

Reports of effects of temperature on TOBEC readings are contradictory. The ambient temperature (T_a) of the equipment during testing was Roby (1991) as a significant factor affecting the TOBEC reading, but Walsberg (1988) and Scott *et al.* (1991) reported that TOBEC reading was not influenced by T_a . Walsberg (1988), Bachman (1994), and Scott *et al.* (1991) reported that body temperature (T_b) was positively correlated with TOBEC readings for a model animal (saline), Belding's ground squirrels, and several species of birds, respectively, and thus influenced estimates of LBM. Roby (1991), however, found that TOBEC reading was not influenced by T_b in bobwhites. Similarly, the presence of metal identification bands may or may not change the TOBEC reading. Scott *et al.* (1991) found that leg bands caused an increase in TOBEC readings independent of LBM in 3 species of birds and, furthermore, that the amount of change was positively related to the size (and possibly the composition) of the

bands. In contrast, Castro *et al.* (1990) and Roby (1991) did not find any influence of leg bands on TOBEC reading.

The fullness of the gut and the hydration status of the animal at the time of testing may influence the TOBEC reading. Bachman (1994) and Voltura and Wunder (1998) reported that gut contents influenced the TOBEC reading in a manner indistinguishable from the influence of the lean mass. In other words, there was no means of determining the gut content-free lean mass of the animal unless the animals were known to have empty guts. Dehydration or electrolyte disturbances alter TOBEC readings (Harrison and Van Itallie 1982, Klish *et al.* 1984, Roby 1991, Walsberg 1988), although Segal *et al.* (1985) and Cochran *et al.* (1989) reported that only extreme electrolyte disturbances in humans resulted in changes in TOBEC readings. Theoretically, TOBEC readings could be affected by changes in water volume and/or electrolyte concentrations because the higher conductivity of lean tissue is due primarily to its greater water and electrolyte content (Harrison and Van Itallie 1982).

My study was undertaken to derive a TOBEC calibration for Richardson's ground squirrels. A second purpose was to evaluate many of the factors that are purported to affect TOBEC readings and, thus, estimates of LBM, and to determine how these factors might be accounted for in this species.

METHODS

Two pregnant female Richardson's ground squirrels were trapped in Granum, Alberta on 8 and 9 April 1997 from private land where animals were to be exterminated.

The 2 animals were housed at the animal care facility in the Department of Biological Sciences at the University of Lethbridge in shoebox cages (38 x 48 x 20 cm), on a bed of aspen shavings, with shredded paper towelling, at an ambient temperature of 18-20°C. Food (LabDiet 5001 Rodent Diet, PMI Nutrition International, Brentwood, Mo.) and water were provided *ad libitum*. Vegetables, fruits, and sunflower seeds were provided as treats every 2 to 3 days. Litters of 7 (5♂:2♀) and 11 (6♂:5♀) were born on 14 April. Neonates were kept with their mothers past weaning at 30 days. At 39 days, the juveniles were housed in pairs in the same conditions, with each mother housed with 1 of her offspring. Housing conditions met or exceeded those required by the Canadian Council on Animal Care (1993).

Effects of Gut Contents and Hydration Status. — To examine the effects of gut fullness on TOBEC, I first needed an approximate rate of digesta passage in this species. I provided sunflower seed dusted with a fluorescent powder to 14 ground squirrels in their home cages for 30 min after depriving them of food overnight (~14 h). I then isolated each animal in similar cages and revisited the cages every 60 min for 18 h. Isolated animals were provided with water and food *ad libitum*. On each visit, I collected all feces in the cage and examined the feces for fluorescence using a handheld ultraviolet lamp. I also frequently moved animals to clean cages after a visit to ensure that no fluorescent powder from one visit was present at the next visit.

Based on this preliminary study (Table 1), I food-deprived 14 Richardson's ground squirrels in the laboratory for 18 h and assumed they then had empty guts. I measured mass and TOBEC (see *TOBEC measurements* below), restored access to food

Table 1. Semi-quantitative analysis of fluorescence in fecal pellets collected at ~1-hr intervals after ingestion by 14 Richardson's ground squirrels of barley coated with fluorescent pigment. Time of ingestion was taken as time of introduction of seed into the home cage after 16-18 h of fasting. Scoring is on a scale where 0 indicates no fluorescence observed, + indicates a barely detectable-by-eye amount, and ++++ indicates a very large amount of fluorescence. Blank indicates no feces collected in the preceding hour for any animal.

Time after ingestion (h)	Fecal fluorescence	
	Average	Range
1		
2	0	0 - ++
3	+	0 - +++
4	+	0 - +++
5	+++	0 - ++++
6	++++	0 - ++++
7	++++	0 - ++++
8	++++	0 - ++++
9	++++	0 - ++++
10	++++	0 - ++++
11	++	0 - ++
12		
13	+	0 - ++
14	0	0 - +++
15	0	0
16	0	0
17	0	0
18	0	0

for 2 h and, assuming they had full guts, measured mass and TOBEC again.

To examine the effect of hydration status on TOBEC, I deprived 16 animals of water for 72 h, measured mass and TOBEC, restored access to water for 3 h, and then measured mass and TOBEC again. Animals were provided with food *ad libitum*.

TOBEC measurement. — Because the 20 animals originally held captive were used in pilot studies and for classroom instruction during 1997-98 and 1998-99, they were not used for TOBEC calibration until spring 1999. Concerned that the captive animals all had very high FBMs as a result of *ad libitum* feeding and limited exercise, I captured 10 additional adult Richardson's ground squirrels from the grounds of the Lethbridge Research Centre (LRC) in spring 1999. I anticipated that these animals would have lower FBMs than the captive animals. Thus, 30 animals (12 ♂, 18 ♀) with a wide range of FBM anticipated were used in TOBEC calibration.

After weighing animals to the nearest 0.1 g on a triple-beam balance, I anaesthetized them by intramuscular injection in the gluteus maximus of ketamine hydrochloride:xylazine (85:15 by volume, dose 100 mg/kg). This dosage was enough to completely immobilize the animal. The anaesthetized animal, with a numbered metal identification tag in each ear (National Band and Tag Co., no. 1 monel self-piercing tag), was subjected to TOBEC measurements using a Small Research Animal Body Composition Analyser (EM-Scan Inc., Springfield, IL, Model SA-2) that had been at an ambient temperature in the laboratory at 20°C for at least 2 hours. The squirrel was placed within the plastic carrier/restraint in a prone position so that the centre of the body was at the centre of the analyser chamber. Body position was further standardized by

tilting the carrier to vertical while holding the upper incisors and allowing the animal's mass to fully extend the body before securing the restraint. After calibration of the SA-2 using the calibration tube provided, I repeatedly introduced the animal/carrier/restraint into the analyser until 10 readings within 0.5% of each other were obtained (excessively divergent readings were discarded). I then also discarded the highest and lowest of the 10 readings and averaged the remaining 8 EM-Scan readings. Tb of the animal was determined using an alcohol thermometer lubricated with petroleum jelly inserted 3-5 cm in the rectum.

Body measurements were taken on the still-immobilized squirrels after removal from the restraint tube. I measured total length (nose to tip of the last vertebra in the tail) to the nearest 1 mm while the animal was firmly pulled to full length. I measured tail length to the nearest 1 mm by placing a ruler against the base of the tail on the dorsal surface and pulling the tail firmly at right angles to the body; I then calculated body length by subtracting tail length from total length. I used a vernier caliper to measure the distal leg segments (from the elbow or knee to the proximal palmar surface) of 1 forelimb and 1 hindlimb, as well as the largest width across the head (zygomatic width), to the nearest 0.1 mm. I used a flexible dressmaker's tape measure to determine the largest mid-abdominal girth while prone to the nearest 1 mm. All body measurements were taken at least 3 times, then averaged. Based on the observed variability in the caliper measurements, averaged limb lengths and zygomatic width were rounded to the nearest 0.5 mm.

Eartags were then removed from 20 of the still-anaesthetized animals, and another

TOBEC reading was taken to determine the effect of metal tags on the TOBEC reading. Anaesthetized animals were then killed in a CO₂ chamber. As animals cooled after death, TOBEC readings were taken when Tb (rectal) was 35, 30, 20, 10 and 4°C for 20 animals. Cooling below 30°C was accelerated by placing the animals in a refrigerator set at 4°C.

Determination of lean body mass was made for all 30 animals. The whole carcass minus gut contents was ground in a manual meat grinder, then dried at 80°C (Kerr *et al.* 1982) until no mass change was noted by repeated measures to the nearest 0.1 g on a triple-beam balance. The entire sample was homogenized in a food processor, and duplicate 3- to 5-g samples were extracted with petroleum ether in a Soxhlet apparatus to remove neutral (non-polar) fats while preserving structural (polar) fats (Dobush *et al.* 1985), so that FBM would represent storage lipids. If necessary, additional samples were extracted until the difference between the proportions of remaining lean mass for 2 duplicate samples was less than 2%. LBM was calculated by multiplying the averaged lean mass per gram of sample by the dry mass of the pre-extraction homogenate, and then adding the mass of water removed by drying. FBM was calculated as the difference between whole body mass (reduced by the mass of the gut contents) and LBM.

Measurements for the same animals with and without eartags, before and after hydration, and before and after gut filling were compared with paired *t*-tests. A stepwise regression of the live TOBEC reading without eartags and body measurements on LBM was then conducted to obtain the regression equation that best predicted LBM. The influence of Tb on TOBEC measurements was assessed separately by regressing the relationship between Tb and the change in the TOBEC reading from 37°C to Tb for each

animal, and then deriving a mean slope and mean intercept to describe the average change. Data are reported as mean \pm 1 SE. JMP-IN statistical software (version 4.0.2 for Windows, SAS Institute) was used for all analyses and figure preparation.

RESULTS

The 30 Richardson's ground squirrels used for TOBEC calibration ranged in total body mass from 188 to 612 g. Soxhlet extractions resulted in the determination of LBMs ranging from 97.0% to 46.8% of body mass, thus spanning the spectrum from animals with virtually no fat reserves to those for which body mass was more than 50% stored fat.

TOBEC readings with and without eartags were highly correlated ($r^2 = 0.98$, Fig. 1), and the eartags were responsible for a small but significant mean increase in TOBEC readings of 9.6 units (1-tailed $t_{19} = 1.89$, $P = 0.037$), which amounted to an inflation of estimates of LBM by 2.5 g. The magnitude of the change in TOBEC reading was independent of sex, age, and size (body mass or LBM) of the animals.

The stepwise regression of TOBEC (for animals with eartags at $T_b = 37^\circ\text{C}$) and the body measurements, as well as several parameters (body mass², girth/length, girth/length², weight/length, and weight/length²) calculated to address differences in body shape and size (Fiorotto *et al.* 1987) on LBM, revealed that the prediction of LBM using TOBEC was best improved by inclusion of forelimb length (adjusted r^2 improved from 0.75 to 0.92). Equation 1 relates TOBEC (at 37°C with eartags) and forelimb length (in mm) to LBM (Fig. 2):

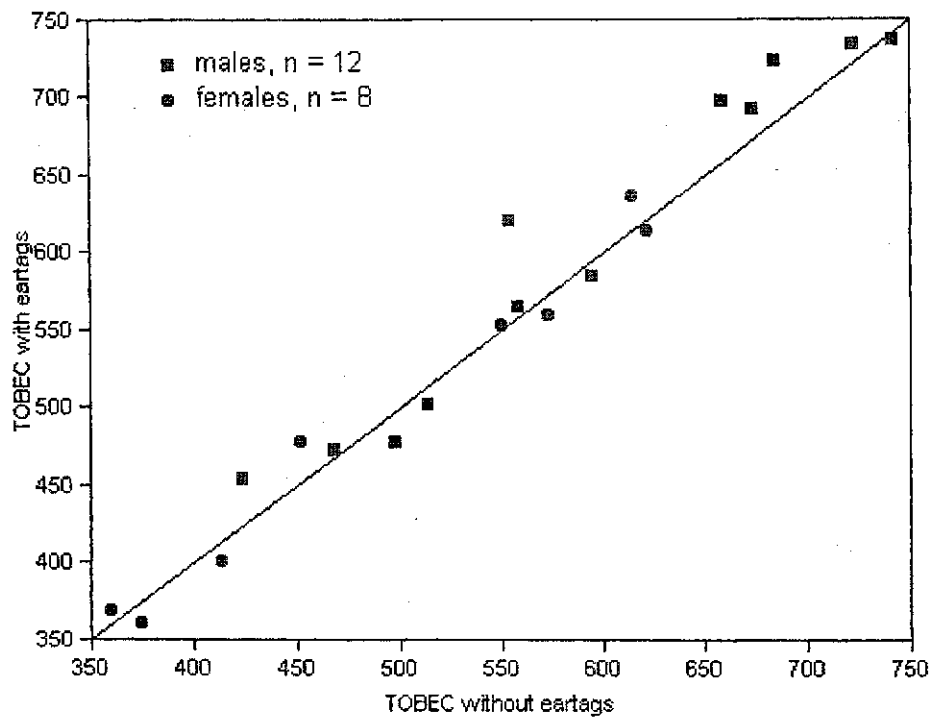


Figure 1. Relationship between TOBEC measurements of 20 Richardson's ground squirrels with and without eartags. The line $Y = X$ is drawn for reference.

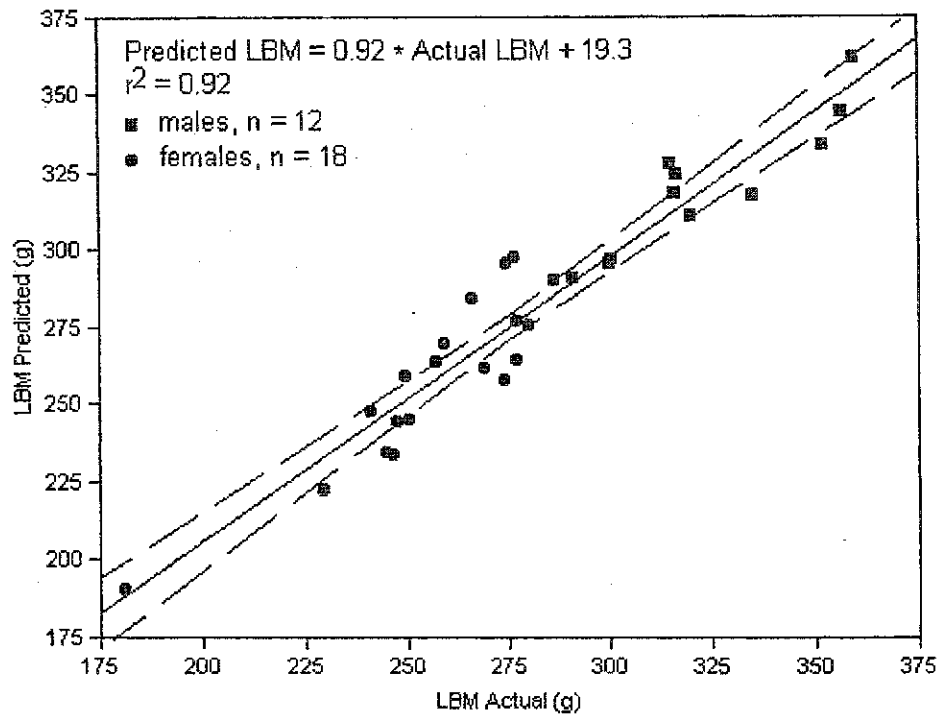


Figure 2. Relationship of actual lean body mass (LBM) determined by fat extraction and predicted LBM determined using TOBEC (at 37°C with eartags) and forelimb length for 30 Richardson's ground squirrels. Dashed lines indicate 95% confidence intervals of the regression slope.

$$\text{LBM} = 0.25 * \text{TOBEC} + 8.16 * \text{Forelimb} - 174.0 \quad (1)$$

$$r^2 = 0.92$$

Body mass is the most repeatable measurement in the field. To examine how much accuracy is gained by using the slightly more difficult measurement of forelimb length in lieu of body mass, I regressed TOBEC and body mass (in g) on LBM. Equation 2 explains 12% less of the variability in LBM than is explained by using forelimb length.

$$\text{LBM} = 0.36 * \text{TOBEC} - 0.12 * \text{Body Mass} + 137.9 \quad (2)$$

$$r^2 = 0.80$$

As Tb declined, the change in TOBEC increased (Fig. 3, $r^2 = 0.61$, $P < 0.0001$). TOBEC measurements made when Tb was not 37°C can be adjusted according to equation 3.

$$\text{Adjusted TOBEC} = \text{TOBEC} + 185.4 - 4.4(\text{Tb}) \quad (3)$$

When adjusted TOBEC was compared to the TOBEC value taken at 37°C (without ear tags), the correction was best for Tb of 35°C ($r^2 = 0.97$) and progressively deteriorated in value as Tb dropped ($r^2 = 0.94, 0.88, 0.85$ and 0.74 for Tbs of 30, 20, 10 and 4°C, respectively).

Body masses of the 14 squirrels in the experiment on gut fullness were 365 - 650 g (529 ± 22.8). Squirrels had masses that were significantly lighter after 18 h of food

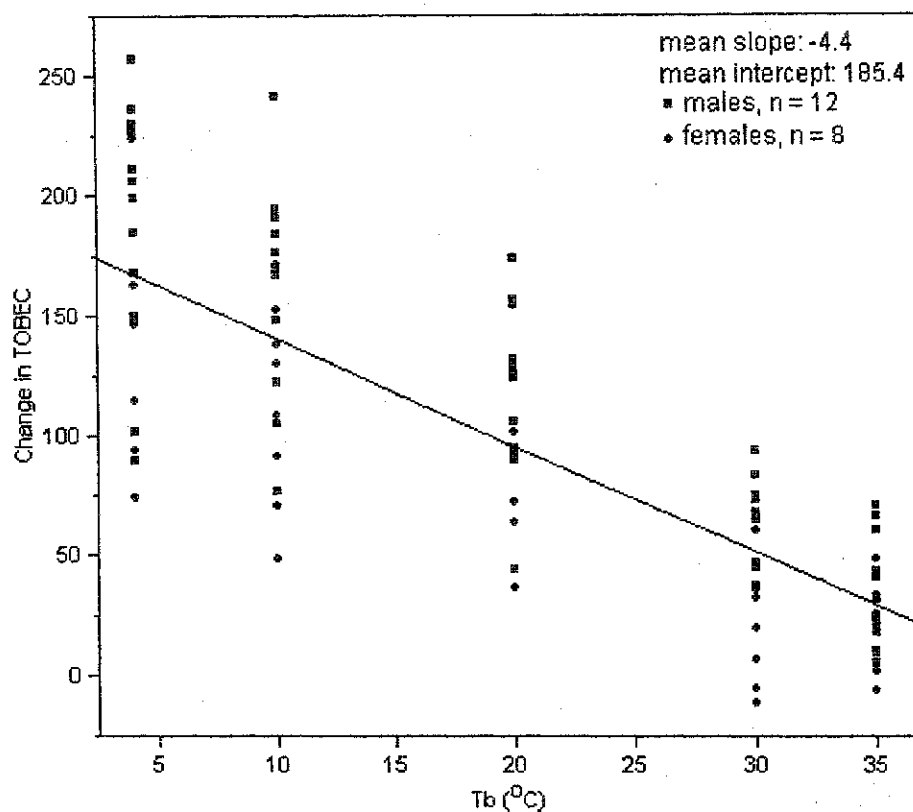


Figure 3. The difference between TOBEC measurements at 37°C and at 5 lower body temperatures (Tb), all for Richardson's ground squirrels with eartags removed. TOBEC measurements decreased as Tb decreased. The solid line is the average of all regressions of 20 animals ($n = 20$ for each Tb) measured at each Tb.

deprivation than after 2 h of access to food (mean difference: 27.7 ± 1.9 g, paired $t_{13} = 14.50$, $P < 0.0001$, Table 2). The increase in mass due to refeeding resulted in a significant increase in the TOBEC reading of 83 ± 8.8 units (from 576 ± 29.6 units to 659 ± 25.8 units, $t_{13} = 9.46$, $P < 0.0001$) which in turn resulted in a significantly increased estimate of LBM by 20.8 ± 2.2 g and a significant increase in the estimate of FBM by 7.0 ± 2.2 g. Thus, the increase in mass as a result of filling the gut was partitioned by TOBEC into both LBM and FBM, although most (75%) of the mass was partitioned in LBM. Because this partitioning was not in the same ratio as FBM/LBM, the FBM as a percent of body mass declined slightly but significantly by $0.9 \pm 0.4\%$ ($t_{13} = 2.25$, $P = 0.042$).

Initial body masses of the 16 squirrels in the experiment on hydration were 365 - 670 g (533 ± 22.3 g). Squirrels were significantly lighter after 72 h of water deprivation than after 3 h of access to water (mean difference: 27.6 ± 4.4 g, $t_{15} = 6.21$, $P < 0.0001$). The resultant change in TOBEC due to rehydration was a significant increase of 104 ± 14.3 units (from 555 ± 31.9 units to 660 ± 23.4 units, $t_{15} = 7.32$, $P < 0.0001$). The estimate of LBM increased significantly by 26.1 ± 3.6 g, but the increase in the estimate of FBM (1.4 ± 3.0 g) was not significant. In this case, almost all of the increase in body mass (95%) due to rehydration was partitioned by TOBEC into LBM and little was partitioned into FBM. Because this partitioning was not in the same ratio as the FBM/LBM, the FBM as a percent of body mass decreased significantly by $1.9 \pm 0.5\%$ ($t_{15} = 4.04$, $P = 0.0011$). Because the increase in mass was partitioned overwhelmingly into LBM, and because water would be measured as conductive by TOBEC, and because the

Table 2. Effects of gut filling and hydration status on body composition in Richardson's ground squirrels. Estimates of LBM and FBM were based on TOBEC measurements. Data are presented as mean \pm SE. Significant differences between adjacent columns based on paired *t*-tests: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS = not significant.

	Gut empty		Gut full		Dehydrated		Hydrated	
<i>n</i>	14		14		16		16	
Body mass (g)	501 \pm 23.3	***	529 \pm 22.8		506 \pm 25.4	***	533 \pm 22.3	
LBM (g)	293 \pm 10.7	***	314 \pm 9.7		285 \pm 11.0	***	311 \pm 9.0	
FBM (g)	209 \pm 17.4	**	216 \pm 18.1		221 \pm 18.3	NS	222 \pm 18.1	
FBM/Mass (%)	40.7 \pm 2.1	*	39.9 \pm 2.2		42.7 \pm 2.0	**	40.8 \pm 2.1	

previous trial on gut filling with food found that the mass increase was partitioned into both LBM and FBM, I reasoned that the changes in LBM with rehydration were due solely to consumption of water and not due to the animals eating food once water was again available.

DISCUSSION

I found that TOBEC readings for Richardson's ground squirrels are influenced by the presence of eartags, T_b , fullness of the gut, and by hydration status. In these experiments, I eliminated the possible influence of T_a and body position by controlling each so that they were uniform for all tests. By using the relatively simple measurement of T_b , I was able to improve the estimate of LBM using TOBEC by adjusting for the small but significant changes in TOBEC reading when T_b was not 37°C. T_b of anesthetized animals varied between 34 and 37°C. By correcting for the lowest temperatures, my estimates of LBM were increased by only 2%, a small but still significant amount. Temperature correction allows TOBEC measurements on dead animals, although low temperatures were associated with increasing variability of TOBEC readings during calibration. The inclusion in the calibration of the effect of metal eartags resulted in a reduction in the estimate of LBM of 2.5 g across the entire range of LBM, which amounts to 1% or less of the corrected LBM.

Forelimb length improved the estimation of LBM using TOBEC to a greater extent than did body mass. Because body mass is not well-correlated with LBM (see Fig. 4, Chapter III), the use of some other measure of body size that is independent of mass is preferable (Morton *et al.* 1991; Unangst and Wunder 2001). In other species,

morphometric measurements similar to those I made may also prove easy to assess and equally valuable in improving the estimate of LBM using TOBEC.

The partitioning of the increase in mass due to feeding on a prepared lab diet into both LBM and FBM raises a new concern when using TOBEC. Bachman (1994) found that the mass of the gut contents of field-living Belding's ground squirrels were incorporated solely into LBM. If the diet of an animal is composed of elements that are differentially conductive, then estimates of both LBM and FBM will be suspect unless the amount of mass in the gut is known. Voltura and Wunder (1998) suggested measuring animals at the same time of day so that the degree of gut fullness between different dates and between individuals would be comparable; however, even this strategy may fail if there is variability in gut capacity, as Voltura (1997) found in prairie voles. Ground squirrels are generally herbivorous and therefore do not likely experience the large differences in mass that carnivores would as a result of gut filling. However, when energy needs are high, such as during lactation, animals may fill the gut to a greater extent to meet those needs, and so the error of estimations of LBM and FBM based on TOBEC could be larger at some stages of the life history than at others. My digesta passage experiment suggests that the gut of Richardson's ground squirrels is cleared about 8 - 10 h post-ingestion, based on the peak in fluorescence observed at that time. Female Richardson's ground squirrels spend 9 - 15 continuous h in overnight sleep sites during the active season (Michener 2002), and are presumed to fast during this time until they emerge in the morning. The minimum sleep time of 9 h would be sufficient to assume that the gut would be empty if ground squirrels were trapped and measured with TOBEC

before they had an opportunity to feed in the morning. Alternatively, animals could be held in captivity and without food until the gut had cleared before TOBEC measurements were taken. Forcing a fast on an animal could be impractical for some researchers, particularly with species that experience undue stress as a result of the fast itself or if being kept absent from the field for long periods, and it seems unnecessary given that many animals voluntarily fast for sufficient periods of time to ensure emptying of the gut. It is therefore most likely that trapping schedules that are timed to such fasting events will preclude concerns about effects of gut fullness on TOBEC.

Dehydration, and its effect on TOBEC readings, may be a concern particularly for animals that have been in hibernation and have thus gone without water, either preformed in food or as free water, for several months. Changes in hydration status of Richardson's ground squirrels were reflected primarily in LBM, with only insignificant changes in FBM. Thus, TOBEC can be used without regard to hydration status if the principle aim of the investigator is to evaluate the FBM, but care must be taken if estimates of LBM are the primary goal of using TOBEC.

Muchlinski and Carlisle (1982) found that golden-mantled ground squirrels (*S. lateralis*) did not experience any significant differences in urine osmolarity during periodic arousals when hibernating when these were compared with active, normally hydrated animals. Bintz and Mackin (1980) found that *S. richardsonii* in short-term (7 day) laboratory experiments adjusted their catabolism of protein relative to adipose tissue to maintain water balance. Animals in hibernation would likely not be severely dehydrated at the end of hibernation because water derived from protein metabolism (the

sum of preformed and oxidative water) would have replaced much of the water lost due to fat metabolism and insensible (mostly respiratory) water losses (Bintz and Mackin 1980). Muchlinski and Carlisle (1982) suggested that water losses due to fat metabolism would be minimal given the low metabolic rates that animals maintain during hibernation, and the low vapour pressure gradient between the animal and the environment of the hibernaculum.

The presence and influence of dehydration have been explored using plasma protein levels and packed cell volume as 2 measures that are supposed to indicate hydration status. Huang and Morton (1976) found that both total plasma protein levels and packed cell volume varied seasonally in a population of Belding's ground squirrel, with high levels of both noted particularly at emergence from hibernation. Yearling and adult yellow-bellied marmots (*Marmota flaviventris*), on the other hand, did not show seasonal differences in packed cell volume (Armitage 1983). The relationships between hydration status (expressed as plasma volume), total protein levels, and packed cell volume have been questioned. Huang and Morton (1976) themselves point out that increases in plasma protein levels at emergence may not be due to decreases in plasma volume but rather to increased erythropoietic activity. The assumption that packed cell volume reflects plasma volume was questioned by Hartman and Morton (1973). They found that packed cell volume was correlated with plasma volume for high altitude-dwelling *S. beldingi*, but not for antelope ground squirrels (*Ammospermophilus leucurus*), a desert-dwelling species uniquely adapted to minimize water losses. The prevalence and degree of dehydration and the need to control for its effect on TOBEC-based estimations

of LBM and FBM need to be investigated further for Richardson's ground squirrels, particularly because they are a xerically-adapted species. This assessment should use methods that more directly measure body water such as by dilution methods (e.g. Bowen and Iverson 1998).

TOBEC is a useful technology for non-destructive determination of body composition, but its calibration is demanding. I endorse the recommendations of the many authors cited herein that a new calibration must be done for each new species. In addition, each new calibration must address every issue which has been shown to influence the accuracy of TOBEC readings. While not all issues will necessarily be relevant on a species-by-species basis, it is clear that many of them can put into question the accuracy of estimates based on this technology.

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Chapter III

EFFECT OF SEED CACHES ON CHANGES IN BODY COMPOSITION OF HIBERNATING RICHARDSON'S GROUND SQUIRRELS (*SPERMOPHILUS RICHARDSONII*)

ABSTRACT

Energy utilization and the influence of seed caches on changes in body composition during hibernation were investigated in Richardson's ground squirrels (*Spermophilus richardsonii*) in southern Alberta, Canada. Body temperature (T_b) was measured by radiotelemetry twice daily during hibernation, ambient temperature (T_a) in the hibernaculum was estimated from soil temperature, and pre- and post-hibernation body composition was measured using TOBEC. Hibernacula were excavated for evidence of seed caches. T_a, T_b, and data on oxygen utilization from Wang (1978, 1979) were used to determine total VO₂, which was used with changes in body composition to predict mass loss. Torpor accounted for 77.3 ± 1.3% of hibernation, but only 4.8 ± 0.6% of total VO₂, whereas intertorpor euthermy comprised 6.5 ± 0.7% of hibernation yet accounted for 43.7 ± 4.7% of total VO₂. The remainder of hibernation was accounted for by periods of post-immersion and pre-emergent euthermy and the times spent arousing from torpor and cooling. Pre-emergent euthermy was longer for squirrels with seed caches (4.0 ± 2.8 days, n = 13) than for those without (0.7 ± 0.1 days, n = 3). Non-caching squirrels (n = 9) lost slightly more than the predicted amount of mass during hibernation, whereas caching squirrels (n = 8) lost 55.7 ± 18.9 g less than predicted. The magnitude of the discrepancy was positively correlated with time in pre-emergence euthermy, indicating that this is probably the time when the cache is

consumed. Caching and non-caching squirrels began hibernation with similar body masses and composition, but the former had higher total tissue mass than expected at emergence. I conclude that seed caches in the hibernaculum subtend recovery of both lean and fat tissue during pre-emergence euthermy, and that caching of seeds in one active season enables males to recoup mass before mating in the next active season. Thus, seed caching can be viewed as a cost of reproduction for males with the potential benefit of improved success in mate competition.

INTRODUCTION

In southern Alberta, Canada, Richardson's ground squirrels (*Spermophilus richardsonii*) hibernate underground for periods of 3-4 months for juvenile (<1 year old) males, 6-7 months for juvenile females, and 8 months for adults (≥ 1 year old) of both sexes (Michener 1979, 1983, 1985, 1992, 1993, 1998; Michener & Locklear 1990a). Hibernation, the period during which squirrels are continuously sequestered underground, encompasses post-immigrant euthermy, heterothermy, and a pre-emergent period of euthermy. During heterothermy, which accounts for the vast majority of hibernation, animals spend most of the time in torpor, conserving energy by allowing body temperature (T_b) to drop to near ambient soil temperatures (T_{soil}) (Michener 1992). However, torpor is interrupted by brief (usually <24 h), energetically expensive arousals to euthermic temperatures, and thus heterothermy is further subdivided into bouts of torpor which last for 4-25 days, punctuated by periods of rewarming, euthermy, and then cooling back to torpid temperatures. Juveniles enter hibernation later than adults of the

same sex, but they emerge from hibernation at the same time. Consequently, juveniles spend less time in hibernation than adults but squirrels of the same sex partition their time in hibernation between torpor and euthermia similarly (Michener 1992). Regardless of age, males spend a smaller proportion of hibernation in heterothermy than females (92.5% vs. 98.2%, Michener 1993). This sexual difference is primarily due to a longer pre-emergence period of euthermia; males spend an average of 9 days (range: 1-25 days) euthermic before emerging from hibernation after their last torpor bout, whereas all females emerge 1-2 days after their last torpor bout (Michener 1992, 1993).

Richardson's ground squirrels hibernate alone in specially prepared chambers which are rarely used for sleeping purposes before hibernation but are occasionally used for a few nights after hibernation (Michener 1992, Chapter IV). Michener (1992, 1993) and I (Chapter IV) noted that some male Richardson's ground squirrels cache seed in the hibernaculum which could be used to supplement the fat reserves that are accumulated before hibernation. Females do not cache seed and so presumably depend solely on fat reserves throughout hibernation. If males use the seed cache as a food source during hibernation, then the rate of mass loss during hibernation should be lower than that of animals without cached seed. Michener (1992, 1993) noted that males lost significantly less mass per euthermic day during hibernation than females (7.0 vs. 9.3 g/day). In particular, males with long (≥ 6 days) pre-emergent periods of euthermia experienced unexpectedly low (< 6 g/euthermic day) mass losses, whereas animals with short (≤ 3.5 days) pre-emergence periods lost mass at > 6 g/day (Michener 1993). The presence of seed caches in the hibernacula of 5 of 6 animals in the former group, and the lack of such

caches in the latter group, led her to infer that the pre-emergence period of euthermy was the period when males might be consuming the seed cache. If this is true, then males that both have a cache and experience a period of euthermy prior to emergence should have lower overwinter weight losses than those that either do not have a cache or do not have a prolonged (several days) period of pre-emergence euthermy.

Weight losses overwinter can be measured for animals that are trapped and weighed both immediately before and after hibernation. Theoretically, estimation of overwinter weight losses can be made if metabolic rates for the period of hibernation are linked quantitatively with the amount of body tissue consumed as the energy source for those rates. Wang (1978, 1979) measured metabolic rates (oxygen consumption, VO_2) for captive Richardson's ground squirrels hibernating at various ambient temperatures (T_a). Each torpor cycle during hibernation was divided into 4 distinct periods: entry into torpor, torpor, arousal, and intertorpor euthermy. Wang found that entry into torpor was regulated so that VO_2 was independent of T_a or T_b , whereas VO_2 during the other 3 periods was dependent on both T_a and T_b . Thus, estimates of energy expenditure during hibernation require measures of both animal and soil temperature, as well as information on allocation of time in hibernation to the 4 periods of the torpor cycle.

Estimates of mass loss based on the seasonal VO_2 expense depend on knowing the proportional contributions of fat and lean body tissue to metabolism during hibernation. This requires knowledge of pre- and post-hibernation body composition. The use of total body electrical conductivity (TOBEC) to determine body composition (lean body mass (LBM) vs. fat body mass (FBM)) allows determination of the ratio of fat to carbohydrate

and protein used as fuel during hibernation, a technique that has been employed successfully in studies of the arctic ground squirrel (*S. parryii*, Buck and Barnes 1999a, 1999b).

This study was undertaken to describe the pattern of energy expense incurred by free-living Richardson's ground squirrels during hibernation and to address the questions of how body composition (LBM vs FBM) changes during that period and what the role of seed caching by males is. I reasoned that for those animals that did not have caches (some $\sigma\sigma$ and all ♀♀), mass losses could be predicted accurately from a knowledge of T_a , T_b , and changes in body composition during hibernation. I predicted that, among males with caches, mass losses would be less than predicted only for males that experienced a period of pre-emergence euthermia during which they used those caches to offset mass losses.

METHODS

Field Study Site and Animal Handling. — Richardson's ground squirrels were studied at a 1.4-ha site located 5 km E and 1 km S of Picture Butte, Alberta, Canada (49°52'N, 112°40'W, elev. 870m). A colony of Richardson's ground squirrels established on this site in 1985 and 1986 has been under study since its inception (Michener 1996). Animals were uniquely identified by ear tags and fur dye, and they were regularly live-trapped (Tomahawk Live Trap Co., single-door, squirrel traps) and weighed to the nearest 5 g (Pesola).

In mid-September 1997, I radiocollared (see *Radiotelemetry and Torpor Cycle*

Partitioning below) 15 juvenile males. One radiocollared animal drowned in a grain hopper prior to immergence, 2 died underground during hibernation of undetermined causes, and 1 radiocollar failed after the animal immerged; the remaining 11 animals were telemetered until emergence in 1998. However, 5 of these 11 provided Tb's that were improbably low (<2°C) and so these 5 animals were excluded from the energy utilization part of the study.

An attempt in 1998-1999 to expand the sample size to include both adult males and females was frustrated by frequent radio failures, underground mortality due to undetermined causes, and underground mortality due to predation by a badger (*Taxidea taxus*) that took up residence on the study site in mid-September of 1998. Ten adults (5 of each sex) were radiocollared in early summer 1998, but none survived to emergence; 3 males were eaten by a badger, 3 animals (2 ♀♀ and 1 ♂) were killed and cached by a badger, and 4 animals (3 ♀♀ and 1 ♂) died underground of undetermined causes. Carcasses of animals that died of undetermined causes were unusable for any element of the study due to missing tissue or decay. Cached carcasses, located by finding the intact radio still around the animal's neck, were recovered by excavation 0, 2, and 10 days after death. Because these 3 carcasses were in good condition, I assumed that no appreciable changes in mass or body composition had occurred after death. Because 1 female carcass was not assessed for TOBEC, only 1 adult male and 1 adult female were used for both parts of the study.

Of 28 juveniles radiocollared in 1998 (6 ♀♀ in August/September and 22 ♂♂ in September/October/November), only 9 (1 ♀ and 8 ♂♂) emerged the following spring. Of

these 9, 1 male could not be located by radiotelemetry during hibernation and so was excluded from the study. Radiocollars failed on 2 other males, resulting in exclusion of these 2 from the energy utilization part of the study, but they were included in the body composition part of the study. Of the other survivors (1 ♀ and 5 ♂♂), improbably low Tb's ($<2^{\circ}\text{C}$) were recorded for the female and 2 males and so these three were also excluded from the energy utilization part of the study, but included in the body composition part of the study. Only 3 juvenile males radiocollared in 1998 were included in both the energy utilization and body composition parts of the study. An additional 3 juvenile males were exhumed early in hibernation to examine mass losses and the potential for utilization of seed caches during the post-immersion period of euthermia. One was exhumed immediately after entering its first torpor (4 days post-immersion) and 2 after entering their second torpor (10 and 11 days post-immersion). These 3 were measured and then released uncollared 2-3 hours after exhumation; 2 emerged the following spring but the fate of the third was not known. Of 16 juveniles that died, 7 (3 ♀♀ and 4 ♂♂) were eaten by a badger and 4 (♂♂) died of undetermined causes, so were excluded from the study. The remaining 5 (2 ♀♀ and 3 ♂♂) were cached by a badger and recovered for study. One male was not assessed for TOBEC and was therefore only used in the energy utilization part of the study. The other 4 cached animals were used in both parts of the study. The sample size (n) for each age and sex class in the 2 parts of the study is summarized in Table 1.

Radiotelemetry and Torpor Cycle Partitioning. — Animals were fitted with

Table 1. Sample size (n) of each age and sex class of Richardson's ground squirrels used for the determination of body composition and overwinter energy utilization. Inclusion in the body composition part of the study required pre- and post-hibernation measures of TOBEC on live animals or undamaged carcasses. Inclusion in the energy utilization part of the study required a complete radiotelemetric record of body temperature during hibernation and pre- and post-hibernation measures of TOBEC. Juvenile animals are those hibernating for the first time (i.e. <1 year old) whereas adults are ≥ 1 year old. Although most animals ($n = 26$ of 28) were used in both parts, 2 juvenile $\sigma\sigma$ were not used for energy utilization due to a failure of radiocollars (see *Field Study Site and Animal Handling*).

	Adult		Juvenile		Total
	♀	♂	♀	♂	
Body composition	1	1	3	23	28
Energy utilization	1	1	2	14	18

temperature-sensitive radiocollars (AVM Instrument Co., Livermore, California, Models SM1-H and G3, and Austech Electronics, Edmonton, Alberta, Model UL81T) that broadcast on unique frequencies in the range 148-149 MHz. A radio receiver (AVM Model LQ-2000) and Yagi 3-element antenna were used to determine the underground location of animals by locating the point at the surface where the signal strength was strongest. Animals were located twice daily at approximately 12-h intervals, and 60-s counts of the radio signal were recorded and converted to T_b using calibration curves derived for each radio. Daily censusing records were used to determine the dates of immergence and emergence, and the hibernaculum was identified as the location where the animal entered torpor and remained until either emergence, exhumation, or capture by a badger. The thermistor of the radiocollar was arranged so that it was against the throat of the animal and thus reflected the surface temperature of the animal. Because animals are curled up during hibernation with the throat apposed to the abdomen (Chapter IV), I assumed that this surface body temperature was a reasonable approximation of body core temperature.

Time euthermic during hibernation was initially calculated as any period of time during which radiotelemetry revealed that the surface body temperature of an animal sequestered in its hibernaculum was $\geq 30^\circ\text{C}$ and was determined from the time that T_b was last $< 30^\circ\text{C}$ to the time before it dropped below 30°C again. Conversely, time torpid was initially calculated as the time when T_b was $< 30^\circ\text{C}$ and was determined as the period between 2 adjacent times euthermic (i.e. from the point before T_b dropped below 30°C to the time before T_b next rose above 30°C again). Because T_b was measured by

radiotelemetry at approximately 12-h intervals, correction factors were applied to these initial calculations to estimate the times required for cooling and rewarming for each torpor cycle. Wang (1973, 1978, 1979) reported that the rate of warming of Richardson's ground squirrels during an arousal averaged $0.174^{\circ}\text{C}/\text{min}$ in field studies (range $0.093 - 0.260^{\circ}\text{C}/\text{min}$), lasted 2-6 h, and was dependent on the T_b from which arousal began. However, no data were presented (Wang 1979) from which to determine the relationship between the rate of warming and T_b at the beginning of an arousal. Therefore, I determined the time required for arousal by dividing the difference between 37°C and T_b before warming began by $0.174^{\circ}\text{C}/\text{min}$. All times for arousal calculated in this manner were between 2 and 6 h. The time for arousal was subtracted from the initial time euthermic to give an adjusted time euthermic. The rate of cooling of Richardson's ground squirrels was $0.017^{\circ}\text{C}/\text{min}$ in field studies regardless of T_a , and it typically took an animal 24-36 h before a stabilized T_b was reached (Wang 1973, 1978, 1979). This rate of cooling was divided by the difference between 37°C and the stabilized T_b reached within 36 h after cooling began to calculate the time of entry into torpor. If the calculated value for time of entry into torpor was less than 24 h, then 24 h was assigned as the time for entry into torpor, otherwise the calculated time was used. In this way, all times for entry into torpor were within the range 24-36 h. This time for entry into torpor was subtracted from the initial time for torpor to give an adjusted time torpid. Thus, time estimates were available for entry into torpor, torpor, arousal, and intertorpor euthermy for each torpor cycle.

Measurement of T_a . — T_{soil} at the depth of the hibernaculum was estimated in both 1997-1998 and 1998-1999 using T_{soil} data at a depth of 50 cm from the Lethbridge Research Centre (LRC) 12 km south of the study site. Animals of all age/sex classes hibernate at similar depths (average 56 cm from the ground surface to the vertical centre of the chamber, Chapter IV; Michener 1992). In October 1998, I buried 2 thermistor probes (YSI Inc., Yellow Springs, Ohio, Model 401) attached to wooden dowels at a depth of 50 cm below the ground surface, with soil packed around the probe tip and along the length of the dowel. I assumed that neither the wooden dowel nor the probe wire conducted appreciable amounts of heat to or from the surface and that any such heat conductance would not interfere with measuring natural changes in soil temperature at that depth. Data were collected using a telethermometer (YSI Inc., Model 42SC) every 48 h for the remainder of the hibernation season. A comparison of data from the LRC and the average of those collected on site in 1998 showed no significant differences between the 2 locations for the period 16 October 1998 to 16 March 1999 (paired $t_{72} = 0.08$, $P = 0.93$, range of differences: -1.9 to 2.3°C), thus validating the use of the LRC data in both the 1997-1998 and 1998-1999 seasons.

Body Composition Measurements. — Pre-hibernation Richardson's ground squirrels were trapped weekly (1997-1998) or biweekly (1998-1999) and taken to an indoor lab adjacent to the study site where the ambient temperature was stable. Animals were either immediately measured or held in plastic shoebox cages (24 by 44 by 20 cm) on aspen shavings for a maximum of 2 h before measurements. After removal of the

radiocollar (if present), animals were weighed to the nearest 0.1 g on a triple-beam balance and then anaesthetized by intramuscular injection in the gluteus maximus of ketamine hydrochloride:xylazine (85:15 by volume, Olson and McCabe 1986). The necessary dosage to completely immobilize animals for 10-15 min (enough time to complete the required measurements) ranged from 25 to 150 mg/kg. Although dosage was tailored to each individual animal's response, there was a trend to lower doses in the post-hibernation (late winter) period and higher doses in the pre-hibernation (summer or autumn) period, though the highest doses (≥ 100 mg/kg) were rarely used.

Tb of the animal was determined using an alcohol thermometer lubricated with petroleum jelly inserted 3-5 cm in the rectum or assumed to be 37°C when not measured. The anaesthetized animal, with a tag in each ear, was then subjected to TOBEC measurements using a Small Research Animal Body Composition Analyser (EM-Scan Inc., Model SA-2). The anaesthetized squirrel was placed within the restraint and the body position was standardized by holding the incisors firmly and letting the animal's own weight extend the body while it was held vertically. The restraint was then closed and placed inside the plastic carrier with the animal prone and the trunk of the body centred in the apparatus. The animal/restraint/carrier was then repeatedly introduced head first into the analyser until 10 readings within 0.5% of each other were obtained (excessively divergent readings were discarded). The highest and lowest of the 10 readings were then also discarded and an average of the remaining 8 EM-Scan readings was recorded. The animal's left distal forelimb segment (from the 'elbow' to the proximal palmar surface) was measured repetitively using a vernier caliper to the nearest

0.1 mm, and these measurements were then averaged. Based on the observed variability in the caliper measurements, averaged forelimb lengths were rounded to the nearest 0.5 mm. Animals were allowed to recover from anesthesia in cages until they were judged fit for release, usually within 90 min, and then returned to the field. Upon release, many animals remained aboveground and resumed feeding or basking, indicating that capture, anesthesia, and TOBEC measurements were relatively unstressful. Because the date of immergence could not be anticipated for a given individual, some animals were subjected to TOBEC measurement multiple (up to 7) times between being radiocollared and immergence, whereas others were only measured once. The last measurement prior to immergence was taken as the pre-hibernation body composition and occurred at a median of 6 days before immergence ($\bar{x} \pm SE = 5.5 \pm 0.6$ days, range 1 - 14, $n = 29$). Because animals experienced only minor mass gains over the 3 weeks preceding entry into hibernation (4.3 ± 0.8 g/week, $n = 22$), I assumed that the variability in the time before immergence when measurements were made did not introduce significant errors. Post-hibernation body composition was determined either on the date of first sighting of the animal aboveground ($n = 22$) or 1-2 days thereafter ($n = 7$).

The pre-hibernation and final LBMs and FBMs were calculated using the TOBEC calibration derived in Chapter II. The last TOBEC measurement of an animal prior to its immergence was used for the pre-hibernation calculations. The final TOBEC reading was defined as the one taken after the animal was exhumed alive ($n = 3$), as the one taken on the whole carcass of an animal after it was recovered from a badger cache ($n = 6$), or as the first one taken after the animal emerged from hibernation ($n = 20$). I subtracted 9.6

from TOBEC readings to compensate for the elevation in the reading due to the presence of ear tags (Chapter II). I then derived an adjusted TOBEC reading to compensate for changes in T_b using equation 1:

$$\text{Adjusted TOBEC} = \text{TOBEC} + 185.4 - 4.4(T_b) \quad (1)$$

LBM was then calculated using equation 2:

$$\text{LBM} = 0.25 * \text{Adjusted TOBEC} + 8.16 * \text{Forelimb (mm)} - 174.0 \quad (2)$$

FBM was calculated as the difference between body mass and LBM, and the proportions of total weight loss due to changes in LBM and in FBM were calculated.

Estimation of VO_2 and Predictions of Mass Loss. — Wang (1978, 1979) did not report the masses of the animals in his studies, so no correction for mass has been attempted in the following calculations. For entry into torpor, the flat cost of 1550 ml O_2 for each cooling event was used, independent of T_a (Wang 1978, 1979). Wang (1978, 1979) presented VO_2 data for the other 3 periods of a torpor cycle, when VO_2 is dependent on T_a , for 4 T_a ranges (-1 - 0°C, 2 - 6°C, 8 - 10°C, and 13 - 15°C). These data were linearly regressed (using the middle of each temperature range as T_a) to allow an estimation of VO_2 at all T_a 's (Equations 3-5).

Torpor: $VO_2 \text{ (ml O}_2\text{/h)} = 4.7 + 0.82 \times Ta,$ (3)

$r^2 = 0.88$

Arousal: $VO_2 \text{ (ml O}_2\text{/arousal)} = 3383.2 - 135.8 \times Ta,$ (4)

$r^2 = 0.98$

Euthermy: $VO_2 \text{ (ml O}_2\text{/h)} = 771.1 - 25.1 \times Ta,$ (5)

$r^2 = 0.64$

Ta was estimated in all three equations as Tsoil at a depth of 50 cm. Equation 4 provided the VO₂ for a complete arousal, whereas equations 3 and 5 were multiplied by the times determined from radiotelemetry to derive VO₂ for torpor and euthermy, respectively. The cumulative VO₂ for each period of all torpor cycles during hibernation was calculated, as was the VO₂ for each of the post-immersion and the pre-emergence periods of euthermy, and then the total VO₂ for the entire period from immersion to emergence was determined.

JMP-IN statistical software (version 4.0.2 for Windows, SAS Institute) was used for all analyses and figure preparation. Data are presented as mean ± SE.

RESULTS

Seed Caches. — The presence or absence of a seed cache was determined by excavation of the hibernacula of 28 Richardson's ground squirrels, described in detail in Chapter IV. Excavations occurred either at exhumation of torpid squirrels ($n = 3$, 4-11 days post-immersion), after death due to predation by a badger ($n = 6$, 15-128 days

post-immersion, 14.2 ± 2.7 days after death), or after the animal had quit the hibernaculum upon emergence ($n = 19$, 54-201 days post-immersion, 15.3 ± 2.7 days after emergence). Because hibernaculum contents were not obtained until the occupant had been sequestered for 4 - 201 days, the amount of seed recovered may have been less than the quantity initially stored. If no trace of any seed was found after a thorough sorting of the contents of the hibernaculum, then I assumed that no seed had been cached. If any seed was present, I assumed the squirrel had a seed cache. Seeds were found in the hibernacula of 16 of 24 males (67%) and 0 of 4 females. Fourteen of 18 surviving males had seed caches with an average mass of 310.0 ± 77.5 (range: 2.6 - 914.2 g) whereas 3 of 6 males that did not complete hibernation had cached seed (2.7, 510.7 and 547.3 g). For the 14 males with a seed cache that survived to emerge, mass of the remaining seed cache did not correlate with the mass of the animal (pre-hibernation or post-hibernation), time in hibernation, or time euthermic either post-immersion or pre-emergence ($P \geq 0.08$ for all regressions). Mass of each male's cache at the start of hibernation was not known. A more complete account of seed caching by this population of Richardson's ground squirrels is made in Chapter IV.

Body Composition. — The pre-hibernation body mass of 28 free-living radiocollared Richardson's ground squirrels ranged from 363 to 622 g (Fig. 1). The pre-hibernation LBMs ranged from 233 to 352 g. Pre-hibernation LBM increased slightly but significantly with increasing body mass. The pre-hibernation FBMs ranged from 74 to 281 g and accounted for 18 to 48% of body mass (mean = $37.3 \pm 1.6\%$, $n = 28$). Before

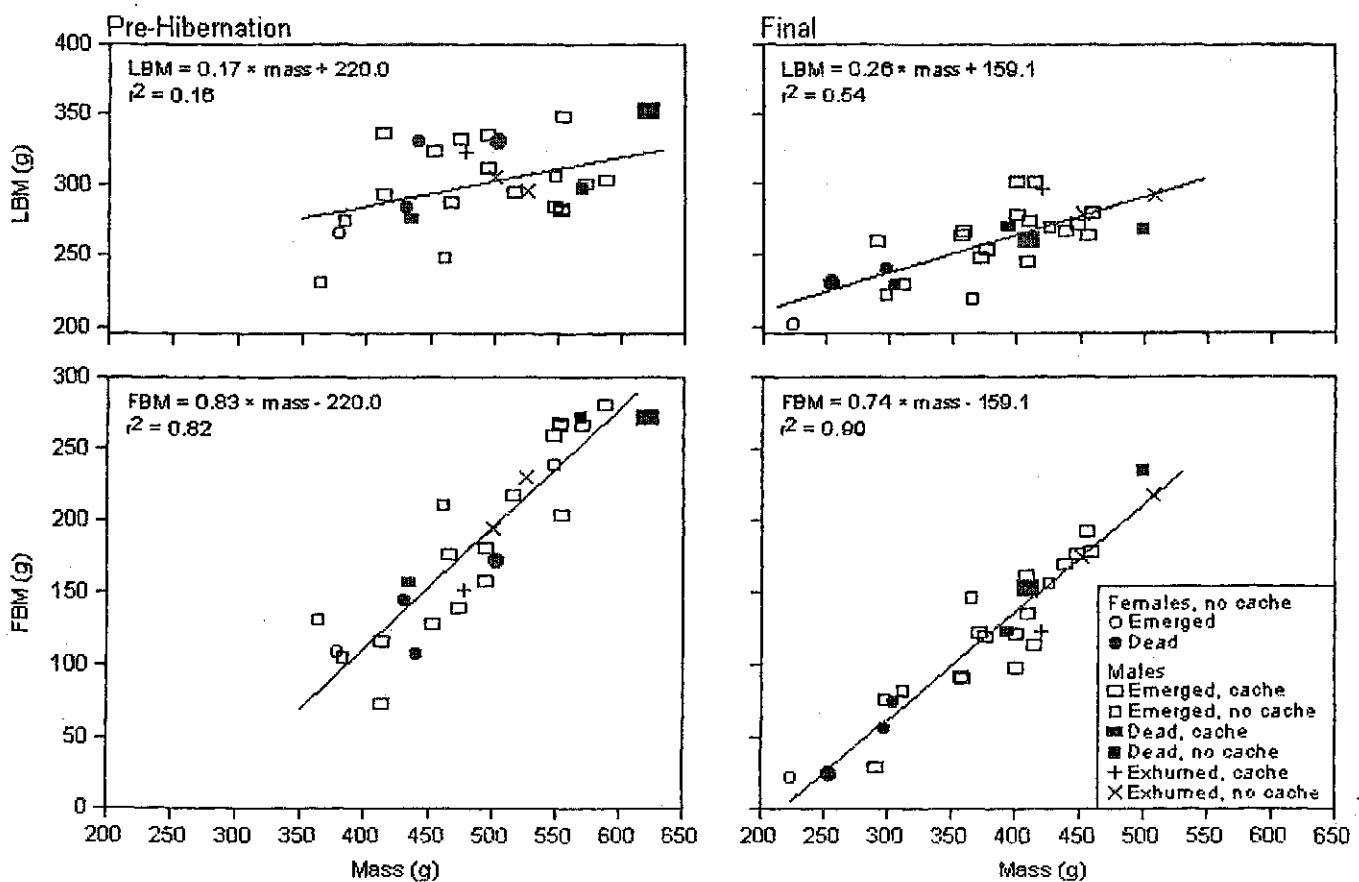


Figure 1. Lean body mass (LBM, upper panels) and fat body mass (FBM, lower panels) related to body mass of 28 Richardson's ground squirrels prior to hibernation (left) and after either emergence, death, or exhumation (right). 6 animals were killed and cached by a badger (*Taxidea taxus*) after 15-128 days of hibernation. 19 animals survived to emerge after 54-201 days of hibernation; 3 males were exhumed alive after beginning their first or second torpor bout (4 - 11 days after immergence). Larger symbols of the same shape and style indicate adults (≥ 1 year old at immergence); all others indicate juveniles. None of 4 ♀♀ and 16 of 24 ♂♂ had evidence of seed caches in their hibernacula.

hibernation, FBMs were more significantly and more positively correlated with body mass ($r^2 = 0.82$, $P < 0.0001$) than were LBMs ($r^2 = 0.16$, $P < 0.05$).

The final body masses of animals, including those that were exhumed alive 4-11 days after immergence into hibernation ($n = 3$), those that were killed and cached by a badger 15-165 days after immergence ($n = 6$), and those that survived hibernation to emerge after 54-201 days of hibernation ($n = 19$), ranged from 223 to 508 g. The final LBMs ranged from 202 to 301 g. Final FBMs ranged from 22 to 234 g, representing a range from 9% to 47% of the total body mass. Both final LBMs and final FBMs were more strongly correlated with body mass than pre-hibernation LBMs and FBMs. The positive correlation of final LBM with final body mass was more significant than the pre-hibernation correlation between the same variables ($P < 0.0001$ vs. $P < 0.05$). The positive correlations of the pre-hibernation and final FBMs with body mass were both strongly significant ($P < 0.0001$). For both pre-hibernation and final body masses, as body mass increased, the proportion of that increase due to an increase in LBM declined, and higher body masses were primarily due to higher FBMs. LBM was not significantly correlated with FBM prior to hibernation, but final LBM was weakly but significantly positively correlated with final FBM (Fig. 2).

For the 17 animals that survived to emerge and for which TOBEC measurements were done, pre-hibernation FBM accounted for 18 - 48% of body mass (mean: $38.0 \pm 2.3\%$) and post-hibernation FBM accounted for 10 - 42% of body mass (mean: $32.0 \pm$

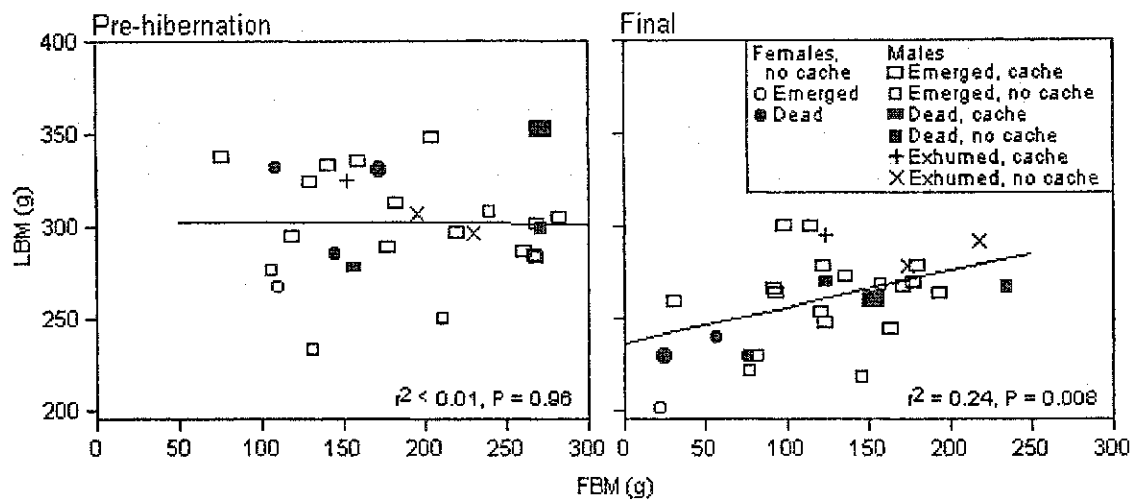


Figure 2. LBM as a function of FBM for 28 hibernating Richardson's ground squirrels pre-hibernation (left) and after either emergence, death, or exhumation (right). Larger symbols of the same shape and style indicate adults (≥ 1 year old at immergence); all others indicate juveniles.

2.0%), a significant decline in FBM of $6.0 \pm 0.7\%$ (paired $t_{16} = 8.70$, $P < 0.0001$). The lone female survivor had the lowest post-hibernation FBM although her pre-hibernation FBM of 29.0% was not unusually low. These changes in FBM in the 17 surviving squirrels amounted to significant overwinter losses of 65.4 ± 6.3 g of fat (paired $t_{16} = 10.3$, $P < 0.0001$), which accounted for $61.3 \pm 4.4\%$ of the total mass lost but only $33.9 \pm 2.1\%$ of fat stores.

Of these same 17 animals, 1 juvenile female and 3 juvenile males had no seed cache. The remaining 13 juvenile males had seed caches. Considering just the juvenile males, the interpretation of mass losses and changes in body composition is rendered somewhat more difficult by the fact that juvenile males with caches spent significantly more time in hibernation, and also spent significantly more days euthermic during hibernation, than those without caches (Table 2). The significant difference in hibernation times was increased by, but not dependent on, the longer duration of pre-emergent euthermy experienced by caching squirrels (i.e., time in hibernation minus time in pre-emergent euthermy was still significantly different between the two groups). However, the significant difference in total time euthermic was dependent on the significantly longer duration of the pre-emergent period of euthermy for caching squirrels (i.e., total time euthermic minus time in pre-emergent euthermy was not significantly different between the two groups).

Pre-hibernation mass, LBM, and FBM were all greater for caching than non-caching animals (Table 2), but these differences were not significant. The same held true after hibernation: mass, LBM, and FBM were all greater for caching animals but the

Table 2. Comparisons of pre- and post-hibernation body composition as determined by TOBEC between hibernating juvenile male Richardson's ground squirrels with ($n = 13$) and without ($n = 3$) seed caches, and the changes that occurred during hibernation. These are the only juvenile males that survived hibernation and for which complete data sets existed. Data are presented as mean \pm SE. Measurements were taken before (Pre) and after (Post) hibernation. % Change is relative to pre-hibernation mass. Significant differences by Mann-Whitney U test between groups ($P < 0.05$) are indicated in bold. LBM = lean body mass, FBM = fat body mass.

		Cache ($n = 13$)	No Cache ($n = 3$)	<i>P</i>
	Hibernation (days)	115 \pm 4.0	86 \pm 10.4	0.031
	Total euthermy (days)	14 \pm 1.1	7 \pm 1.2	0.016
	Pre-emergence euthermy (days)	4.0 \pm 0.8	0.7 \pm 0.1	0.019
Mass	Pre (g)	509 \pm 15.8	464 \pm 47.9	0.23
	Post (g)	398 \pm 13.4	363 \pm 36.9	0.42
	Change (g)	111 \pm 7.1	101 \pm 11.3	0.50
	% Change	22 \pm 1.3	22 \pm 0.4	0.69
	Rate (g/day hibernation)	1.0 \pm 0.06	1.2 \pm 0.02	0.14
	Rate (g/day euthermy)	9.0 \pm 1.12	15.6 \pm 1.77	0.044
LBM	Pre (g)	309 \pm 6.3	278 \pm 16.7	0.14
	Post (g)	267 \pm 4.0	236 \pm 16.2	0.14
	Change (g)	42 \pm 6.9	42 \pm 6.8	0.79
	% Change	13 \pm 1.9	15 \pm 2.4	0.69
	Rate (g/day hibernation)	0.4 \pm 0.05	0.5 \pm 0.14	0.23
	Rate (g/day euthermy)	3.4 \pm 0.64	7.2 \pm 2.65	0.08
FBM	Pre (g)	200 \pm 18.5	185 \pm 40.6	0.69
	Post (g)	131 \pm 12.6	126 \pm 25.2	0.79
	Change (g)	70 \pm 7.1	59 \pm 15.7	0.50
	% Change	35 \pm 2.6	31 \pm 2.0	0.35
	Rate (g/day hibernation)	0.6 \pm 0.07	0.7 \pm 0.12	0.79
	Rate (g/day euthermy)	5.6 \pm 0.88	8.4 \pm 0.90	0.06

differences were not significant. Rates of mass loss, LBM loss, and FBM loss did not differ when expressed per day in hibernation but the rate of mass loss per day euthermic was significantly lower for caching squirrels than for squirrels without caches. The rates of LBM and FBM loss per euthermic day did not differ, although they too were lower for squirrels with caches. The lower rate of mass loss per euthermic day and the greater number of pre-emergence euthermic days, and the larger post-hibernation LBMs but not FBMs suggest that caching animals recouped mass by eating part of the cache and that the cache was consumed during the pre-emergence euthermic period. Caching squirrels were able to remain underground longer and yet still maintain a 10% mass advantage over their non-caching counterparts.

Total mass losses of all squirrels correlated significantly and positively with time in hibernation (Fig. 3, $P < 0.0001$), but the proportion of the total mass loss attributed to a decline in FBM did not relate to time spent in hibernation (Fig. 4, $r^2 < 0.01$, $P = 0.78$). There were no discernible trends to relate the proportional loss of either FBM or LBM to sex or age of the squirrels, but low n for both females and adults precluded statistical testing.

Prediction of Hibernation Mass Loss. — Of the 18 Richardson's ground squirrels for which complete radiotelemetric records existed, 9 juvenile males survived to emerge from hibernation, whereas only 1 juvenile female and no adults of either sex completed hibernation. The 9 surviving juvenile males spent an average of 114.0 ± 4.0 days in hibernation and consumed an estimated 274.5 ± 19.5 L O₂ during that time (Table 3).

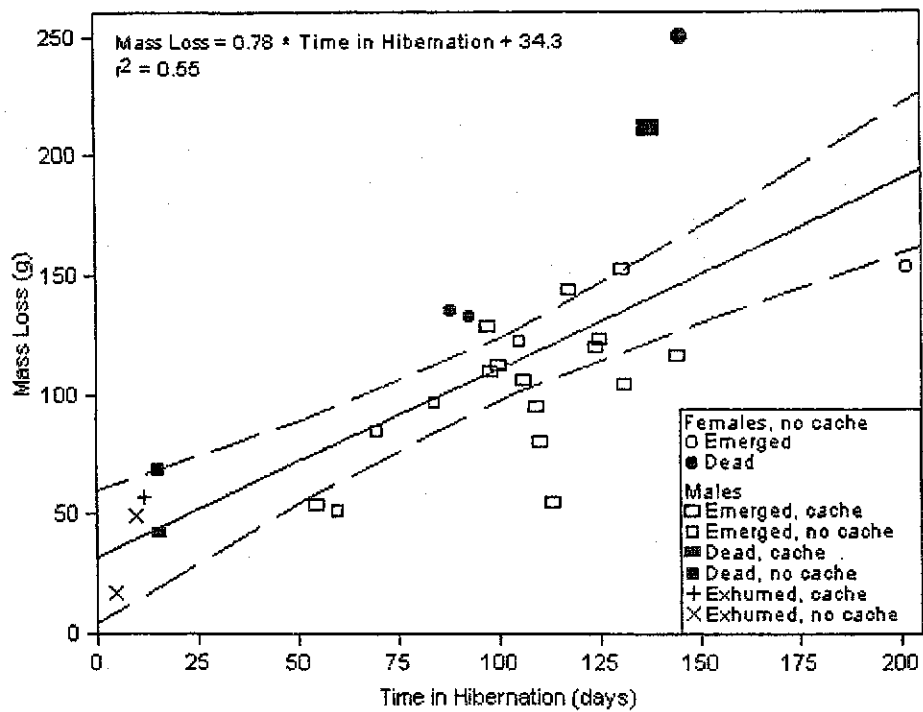


Figure 3. Mass losses during hibernation of 28 Richardson's ground squirrels for which pre- and post-hibernation TOBEC readings were available. 11 squirrels had no evidence of a seed cache in the hibernaculum (all of 4 ♀♀, 7 of 24 ♂♂), whereas 17 did have such evidence (exclusively males). Solid line shows the linear regression for all animals ($P < 0.0001$). Dashed lines show 95% confidence intervals of the regression slope. Larger symbols of the same shape and pattern indicate adults.

Table 3. Mean \pm 1 SE (range) duration and oxygen utilization (VO_2) during different periods of hibernation for 9 radiocollared juvenile male Richardson's ground squirrels in 1997-1998 and 1998-1999 that survived to emergence. Ranges are shown in parentheses. VO_2 was estimated from measures of time partitioning and body temperature determined by radiotelemetry, soil temperature, and data from Wang (1978, 1979) - see Methods for details. Hibernation is divided into the periods of post-immersion euthermy, heterothermy, and pre-emergence euthermy. The period of heterothermy is further subdivided into four periods: entry into torpor, torpor, arousal, and inter-torpor euthermy. Totals in some columns are not exactly 100% due to rounding errors.

Period of hibernation	Duration			VO_2		
	days	% of hibernation	% of heterothermy	L O ₂	% of hibernation	% of heterothermy
Post-immersion euthermy	2.6 \pm 0.6 (0.0 - 6.0)	2.4 \pm 0.6 (0.0 - 6.2)		32.6 \pm 8.2 (0.0 - 77.8)	10.9 \pm 2.4 (0.0 - 24.1)	
Heterothermy:						
Entry into torpor	11.0 \pm 0.6 (8.4 - 14.4)	9.6 \pm 0.4 (8.4 - 12.3)	10.2 \pm 0.4 (8.8 - 12.8)	14.1 \pm 0.9 (10.9 - 18.6)	5.3 \pm 0.4 (3.5 - 7.2)	8.4 \pm 0.5 (6.6 - 10.5)
Torpor	88.3 \pm 3.9 (71.0 - 105.7)	77.3 \pm 1.3 (71.3 - 81.4)	81.9 \pm 1.0 (74.2 - 84.5)	12.3 \pm 0.9 (8.4 - 16.5)	4.8 \pm 0.6 (2.6 - 7.3)	7.5 \pm 0.7 (4.0 - 10.9)
Arousal	1.1 \pm 0.1 (0.8 - 1.5)	1.0 \pm 0.1 (0.7 - 1.2)	1.0 \pm 0.1 (0.8 - 1.3)	27.9 \pm 1.4 (22.3 - 36.0)	10.5 \pm 0.8 (7.3 - 13.7)	16.6 \pm 0.8 (13.3 - 21.2)
Intertorpor euthermy	7.4 \pm 0.8 (5.3 - 13.0)	6.5 \pm 0.7 (4.8 - 11.2)	6.9 \pm 0.7 (5.3 - 11.7)	116.5 \pm 12.2 (90.4 - 206.2)	43.7 \pm 4.7 (25.9 - 68.5)	67.4 \pm 1.8 (60.6 - 75.9)
Pre-emergence euthermy	3.6 \pm 1.0 (0.4 - 9.6)	3.2 \pm 0.9 (0.4 - 8.7)		71.1 \pm 17.0 (11.5 - 172.1)	24.8 \pm 4.8 (4.9 - 49.4)	
Totals	114.0 \pm 4.0 (97.6 - 130.3)			274.5 \pm 19.5 (200.0 - 348.3)		

Heterothermy lasted 107.7 ± 4.4 days, representing $94.4 \pm 1.2\%$ of hibernation. Torpor composed $77.3 \pm 1.3\%$ of hibernation and $81.9 \pm 1.0\%$ of heterothermy, yet accounted for only $4.8 \pm 0.6\%$ of the total VO_2 . The periods of intertorpor euthermia cumulatively accounted for only $6.5 \pm 0.7\%$ of hibernation but represented the most energetically expensive period of hibernation with the consumption of $43.7 \pm 4.7\%$ of the oxygen used during hibernation. Similarly, the periods of post-immersion and pre-emergence euthermia represented only $2.4 \pm 0.6\%$ and $3.2 \pm 0.9\%$ of the time in hibernation yet accounted for $10.9 \pm 2.4\%$ and $24.8 \pm 4.8\%$, respectively, of the VO_2 during hibernation.

I used VO_2 during hibernation to predict mass loss based on the liberation of 20.29 kJ/L O_2 (Wang 1978) and the assumptions that the drop in LBM was due to a combined mobilization of proteins and carbohydrates that yielded 17 kJ/g , and that the drop in FBM contributed 37 kJ/g (Mathews and van Holde 1996). I used equation 7 to convert VO_2 into a prediction of mass loss. Proportional losses of LBM and FBM were available for 26 animals with TOBEC records.

$$\text{Predicted Mass Loss (g)} = \frac{\text{Total VO}_2 (\text{L O}_2) * 20.29 (\text{kJ/L O}_2)}{[\text{pLBM} * 17 (\text{kJ/g})] + [\text{pFBM} * 37 (\text{kJ/g})]} \quad (7)$$

where Total VO_2 is the sum of all the estimated VO_2 for the time the animal was alive underground after immersion, 20.29 is the energy conversion from litres of oxygen to kilojoules, pLBM and pFBM are the proportions of total mass lost due to changes in LBM and FBM respectively as determined using TOBEC, and 17 and 37 are the gram

energy equivalents for LBM and FBM respectively.

To assess the accuracy of these predictions of mass loss, I compared them to the actual mass losses for 7 animals (4 ♂♂, 3 ♀♀) that had no seed cache in the hibernaculum and therefore presumably had no means of offsetting their mass losses overwinter via consumption of food. For these non-caching animals, mass loss was slightly but not significantly under-predicted by 13.6 ± 6.9 g (two-tailed paired $t_6 = 1.98$, $P = 0.10$) and there was a significant correlation between the two (Fig. 5, $r^2 = 0.94$, $P = 0.0003$).

For ground squirrels that did have seed caches (11 ♂♂), I expected that animals would lose less mass than predicted using estimates of VO_2 because they would have consumed some part of the seed cache. Actual mass loss for seed caching animals was indeed significantly less than predicted mass loss (1-tailed paired $t_{10} = 2.95$, $P = 0.01$). Actual and predicted mass losses were not significantly correlated ($P = 0.10$). On average, animals were 55.7 ± 18.9 g heavier than predicted (range: -18 to 180 g). Two animals that had seed caches but did not complete hibernation (1 was exhumed 11 days post-immersion, the other was killed by a badger 14 days post-immersion) were among the 3 animals for which the animal's final mass was actually lighter than or almost equal to predicted. Reasoning that these 2 animals had not had much time to consume the seed cache and had not done so as evidenced by their lighter than predicted body mass, I added these 2 animals to the group that had no seed caches to create a category of animals that either had no cache or no opportunity to consume a cache and re-analysed the actual vs. predicted mass loss data. This time, mass loss was under-predicted by slightly less (12.4 ± 5.5 g) and was still not quite significant (2-tailed paired $t_8 = 2.25$, $P = 0.05$) and

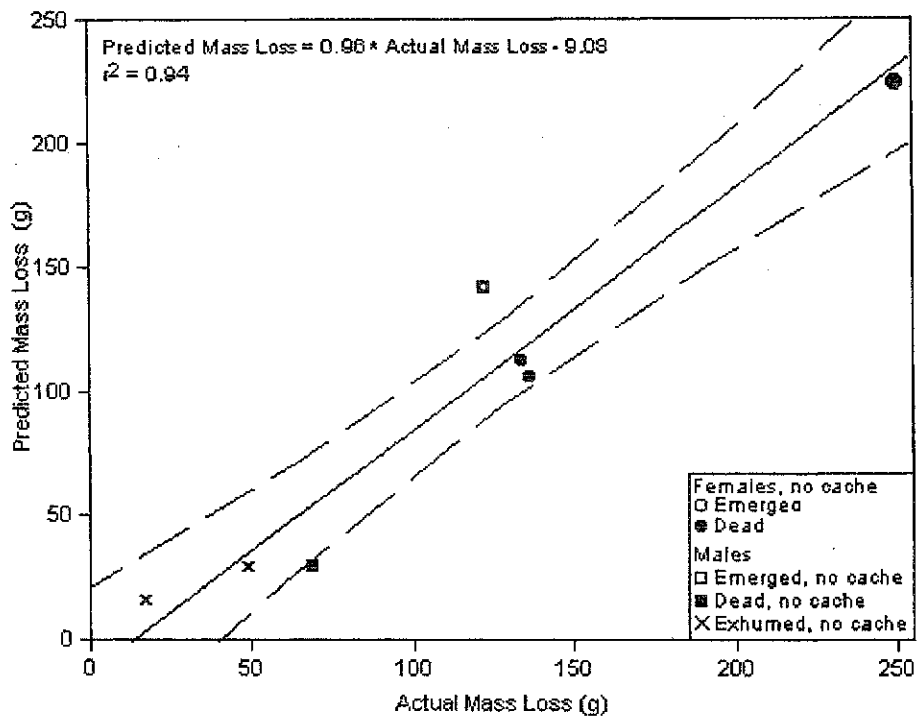


Figure 5. Predicted versus actual mass loss in 7 Richardson's ground squirrels (4 ♂♂, 3 ♀♀) that did not have seed caches in the hibernaculum. Solid line shows the linear regression ($P = 0.0003$). Dashed lines show the 95% confidence intervals for the regression slope. Larger symbols of the same shape and pattern indicate adults.

the correlation between the two improved slightly (Predicted mass loss = $0.95 * \text{actual mass loss} - 7.8$, $r^2 = 0.95$, $P < 0.0001$). By contrast, mass loss was significantly over-predicted for those animals that had a cache and had opportunity to use it (difference: 69.9 ± 20.0 g, 1-tailed paired $t_8 = 3.49$, $P = 0.008$). I compared the magnitude (absolute value) of the difference between the actual and predicted masses for animals with seed caches and those that either did not have caches or had no opportunity to use them and found a significant difference between the two groups ($t_{16} = 2.77$, $P < 0.01$). In other words, the accuracy of my prediction of mass loss during hibernation was significantly better for animals that I presumed did not consume food while in hibernation than for those that had the opportunity to consume food.

If the period of pre-emergence euthermy at the end of hibernation is when the seed cache is consumed, then I predicted that the discrepancy between predicted and actual mass loss for the 8 animals that had seed caches and did complete hibernation would not correlate with the total time in hibernation, but would positively correlate with the time spent in pre-emergence euthermy. The discrepancy in mass loss predictions was not significantly correlated with time in hibernation ($r^2 = 0.08$, $P = 0.49$). The difference between predicted and actual mass loss was, as hypothesized, significantly and positively correlated with the amount of time an animal spent in pre-emergence euthermy (Fig. 6, $P = 0.05$).

For 9 animals (7 that had no seed cache and 2 that I reasoned had not consumed their cache), the rates of total mass loss were 3.0 ± 0.5 g/day of hibernation and 15.3 ± 2.4 g/day of euthermy during hibernation. The average rates of FBM loss were 1.7 ± 0.3

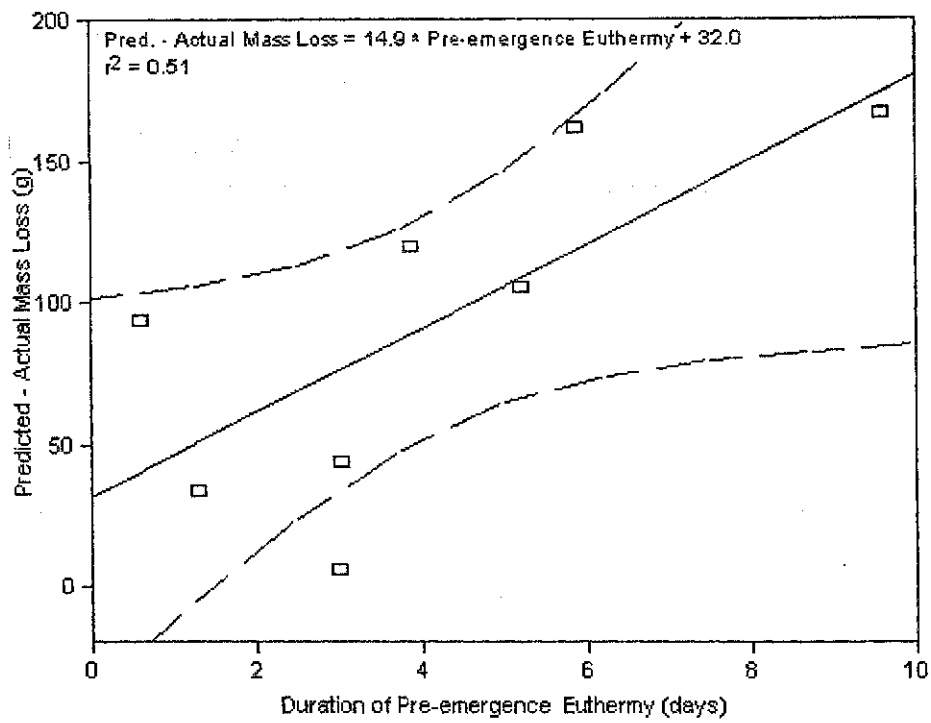


Figure 6. The difference between predicted and actual mass loss in 8 juvenile male Richardson's ground squirrels that survived hibernation and had seed caches, as a function of the amount of time spent euthermic at the end of hibernation prior to emergence. Solid line shows the linear regression ($P < 0.05$). Dashed lines show the 95% confidence intervals of the regression slope.

g/day of hibernation and 8.7 ± 1.6 g/day of euthermy.

When compared to the group with no seed cache or no opportunity to consume a cache, the 9 males that did have seed caches and completed hibernation lost mass at a significantly reduced rate of 1.0 ± 0.1 g/day of hibernation ($t_{16} = 3.92$, $P < 0.002$) and the rate of 8.3 ± 1.1 g/day of euthermy was significantly lower ($t_{16} = 2.20$, $P < 0.05$). The rate of FBM loss was also significantly reduced to 0.6 ± 0.1 g/day of hibernation ($t_{16} = 3.48$, $P < 0.005$), but there was no significant change in the rate of FBM loss per euthermy day (5.1 ± 0.6 g/day of euthermy, $t_{16} = 2.11$, $P > 0.05$). Because the pre-emergence period of euthermy has been implicated as the time of cache consumption by Michener (1992, 1993), the rate of mass gain during that time was estimated by calculating the difference between the predicted mass and the actual mass divided by the time spent in pre-emergence euthermy. Animals with seed caches gained total mass during pre-emergence euthermy at the rate of 38.3 ± 18.4 g/day, of which 26.3 ± 13.5 g/day was due to increases in FBM.

Despite the differences in rates of mass loss between animals that did not have or use caches and those that did, the mass of the lone non-caching male that emerged (425.6 g) was not much different from that of 9 caching males that emerged (409.6 ± 10.9 g). The 9 squirrels that had no cache or had not had time to consume it did not have different emergence masses (487.3 ± 17.7 g vs. 532.1 ± 19.8 g, $t_{16} = 1.69$, $P = 0.11$) than the 9 caching males, they did not lose significantly more mass (94.1 ± 24.6 g vs. 121.2 ± 15.0 g, $t_{16} = 0.95$, $P = 0.36$), and the mass losses expressed as a percentage of the pre-emergence mass were not different ($19.5 \pm 5.1\%$ vs. $22.3 \pm 2.1\%$, $t_{16} = 0.50$, $P = 0.62$).

I reasoned that a smaller remnant seed cache could indicate that seed had been consumed by the squirrel and so smaller caches might be associated with animals that were heavier than predicted. I examined the size of seed caches relative to both the discrepancy between the actual and predicted masses and the actual mass loss for the 8 surviving animals that had not lost as much mass as predicted. There was no significant correlation between the discrepancy in body mass and the size of the remaining seed cache ($r^2 = 0.23$, $P = 0.22$) but mass loss was significantly and positively correlated with the size of the seed cache remaining at the end of hibernation (Fig. 7, $r^2 = 0.64$, $P = 0.02$). In other words, remaining seed caches were smaller for animals that had lost the least mass, presumably indicating that they had replenished mass by consuming food stores.

DISCUSSION

The use of TOBEC and radiotelemetry to estimate body composition and to partition hibernation into euthermia and torpor allowed me to predict the overwinter mass losses of free-living Richardson's ground squirrels. The prediction of mass loss accurately estimated actual mass loss for animals that did not have a seed cache or that had no opportunity to consume the cache. The success of the prediction for those squirrels confirmed the use of T_b and T_a data and Wang's metabolic rates (1978, 1979) at different phases of hibernation to estimate total metabolic expense of the animals, as well as the use of TOBEC to estimate the contributions of FBM and LBM to metabolism during hibernation. In contrast, the predicted mass loss overestimated actual mass loss for ground squirrels that survived hibernation and had a seed cache. Furthermore, the discrepancy between predicted and actual loss of mass overwinter was positively

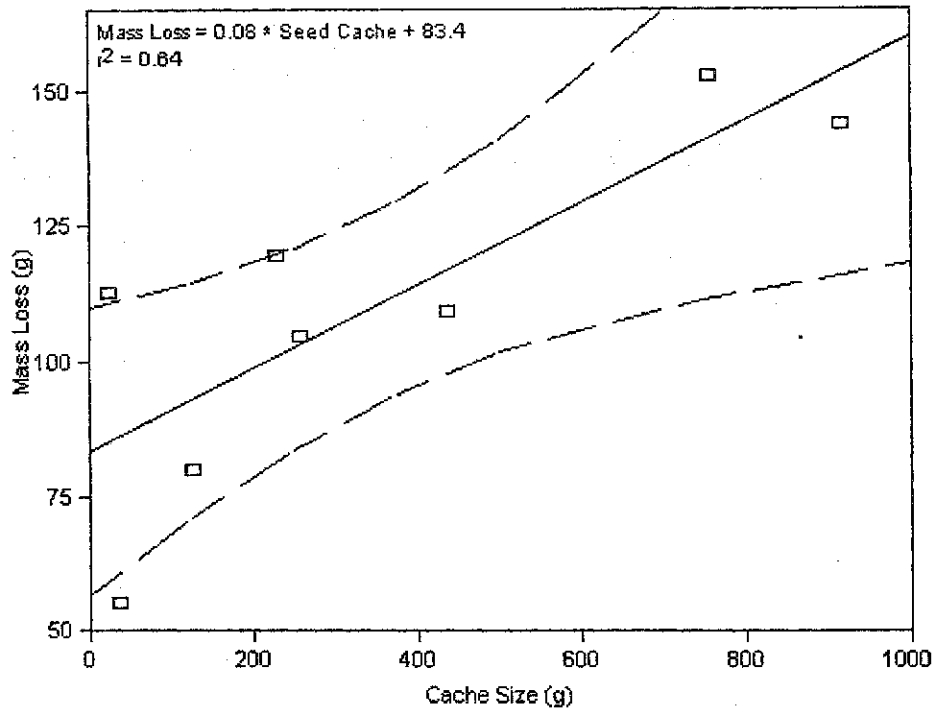


Figure 7. Total mass loss during hibernation as a function of the size of the seed cache remaining after hibernation in 8 juvenile Richardson's ground squirrels that completed hibernation. Solid line shows the linear regression ($P = 0.017$). Dashed lines show the 95% confidence intervals of the regression slope.

correlated with the duration of pre-emergence euthermy, supporting Michener's (1993) hypothesis that the timing of consumption of the cache is at or near the end of hibernation.

Richardson's ground squirrels that had seed caches and had completed hibernation lost mass at rates that were similar to the rates of 21 juvenile males reported for the same study site by Michener (1993, 0.84 ± 0.01 g/day of hibernation, $t_{32} = 1.59$, $P = 0.10$). However, 13 squirrels without seed caches or that I judged had not had time to consume the cache lost mass at rates significantly higher than those reported by Michener (1993, $t_{32} = 4.59$, $P < 0.001$). Michener (1993) did not separate rates of mass loss over the period of hibernation for caching and non-caching squirrels, but did report that 77% of excavated hibernacula of 13 males contained caches and that there was a negative correlation between the duration of pre-emergence euthermy and the amount of mass lost. Michener's (1993) rates of mass loss probably indicate that many of the 21 males had used seed caches, and so had lower rates of mass loss, as has been shown in the present study.

Body mass and composition changes have previously been reported in 3 other species of ground squirrels. Juvenile male Richardson's ground squirrels that completed hibernation had immergence and emergence masses larger than those of adult *S. townsendii* of both sexes (Rickart 1982) and adult *S. beldingi* (Morton 1975), and smaller than all 4 age and sex classes of *S. parryii* (Buck and Barnes 1999a, Table 4). Although juvenile male *S. richardsonii* were in hibernation for less time than all age and sex classes of the other species, their rates of loss of mass and FBM were among the highest, and

Table 4. Time to complete hibernation and changes in body mass and composition in 4 species of ground squirrel (*Spermophilus*): *S. townsendii* - Rickart (1982); *S. beldingi* - Morton (1975); 16 juvenile male *S. richardsonii* from the current study that completed hibernation; *S. parryii* - Buck and Barnes (1999a), males shown are only those that were reproductive after emergence. Species and cohorts are presented in increasing order of whole body mass. Rates of losses are per day in hibernation, % losses are proportions of immergent masses, and mass-specific rates are relative to emergence mass. j = juvenile, a = adults, m = male, f = female, Elev. = elevation, Lat. = latitude.

	Elev. (m)	Lat. (°N)	Hibernation (days)	Immerge (g)	Whole Body Mass			Emerge (g)
					Loss			
					(g)	(g/day)	(%)	
<i>S. townsendii</i> af	1300	41	270	173	80	0.30	46	93
<i>S. townsendii</i> am	1300	41	270	260	132	0.49	51	128
<i>S. beldingi</i> a	3020	38	275	375	140	0.51	37	235
<i>S. richardsonii</i> jm no seed cache (n = 3)	870	50	86	464	101	1.18	22	363
<i>S. richardsonii</i> jm with seed cache (n = 13)	870	50	115	509	111	0.98	22	398
<i>S. parryii</i> jf	820	69	236	607	184	0.78	30	423
<i>S. parryii</i> af	820	69	240	851	277	1.15	33	574
<i>S. parryii</i> jm	820	69	215	733	89	0.41	14	644
<i>S. parryii</i> am	820	69	220	947	25	0.11	3	922

Table 4 (Continued):

	LBM									FBM						
	Immerge (g) (%)		Loss			Emerge (g) (%)		LBM Loss/ Total Mass Loss (%)	Immerge (g) (%)		Loss			Emerge (g) (%)		FBM Loss/ Total Mass Loss (%)
			(g)	(g/day)	(%)						(g)	(g/day)	(%)			
<i>S. townsendii</i> af	143	83	61	0.23	43	82	89	76	30	17	19	0.07	64	11	11	24
<i>S. townsendii</i> am	191	73	67	0.25	35	124	97	51	69	27	65	0.24	94	4	3	49
<i>S. beldingi</i> a	281	75	66	0.24	23	215	92	47	94	25	74	0.27	79	20	8	53
<i>S. richardsonii</i> jm with seed cache (n = 13)	309	61	42	0.36	13	267	67	37	200	39	70	0.62	35	131	32	63
<i>S. richardsonii</i> jm no seed cache (n = 3)	278	60	42	0.49	15	236	65	44	185	39	59	0.66	31	126	34	56
<i>S. parryii</i> jf	471	78	105	0.44	22	366	86	57	136	22	79	0.33	58	57	14	43
<i>S. parryii</i> af	515	61	99	0.41	19	416	73	36	336	39	178	0.74	53	158	27	64
<i>S. parryii</i> jm	566	77	84	0.39	15	482	75	94	167	23	5	0.02	3	162	25	6
<i>S. parryii</i> am	683	72	-12	-0.05	-2	695	75	†	264	28	37	0.17	14	227	25	†

† Small gains in LBM offset part of the mass lost in adult male *S. parryii*, rendering these ratios meaningless.

these resulted in absolute mass and FBM losses that were intermediate among those of the other 3 species. *S. richardsonii* had the highest proportional FBM at immergence, and used proportionally more fat than the other 3 species, consuming about 34% of pre-hibernation FBM over hibernation.

Other than male *S. parryii*, juvenile male *S. richardsonii* committed the lowest proportion of their LBM (about 14%) to overwinter metabolism. Pre- and post-hibernation FBMs and LBMs were not significantly different between caching and non-caching Richardson's ground squirrels. However, the shorter duration of hibernation for the 3 males that did not cache makes these figures misleading. Non-caching squirrels lost FBM and LBM at higher rates than squirrels with caches. If those rates were extrapolated over the longer duration of hibernation experienced by the caching animals, then losses of mass, FBM, and LBM would have been significantly larger for non-caching animals when compared to caching animals. Because there is no reason to expect either group of animals to lose mass faster during most of hibernation, presumably the lower rates of loss for caching animals represent arithmetic combinations of losses before termination of the final torpor bout and gains during the pre-emergence period of euthermia. The gain in mass indicated by the discrepancy between predicted and actual mass losses for squirrels with seed caches is explained by a partial recovery of both FBM and LBM in juvenile male Richardson's ground squirrels. Buck and Barnes (1999b) found that adult male *S. parryii* recouped lean mass by consumption of a food cache and that both adult and juvenile males recouped FBM, whereas non-caching females experienced losses in both FBM and LBM.

Because male Richardson's ground squirrels are almost immediately faced with the task of mate acquisition after emergence (Michener and Locklear 1990a), increased LBM would confer advantages of strength and size in the male-male competition for mates and increased FBM would subsidize the energy costs of mating activity. Males deplete FBM as the substrate for the high energy costs incurred during mating (Michener and Locklear 1990a). Michener (1992) suggested that energetic costs of mating could be better met if the seed cache was used to recover FBM lost during hibernation. In this study, because of the differences in time spent in hibernation and the small sample size for non-caching males, recovery of FBM was not significant. Clearly, a larger sample size of non-caching males to compare with caching males would provide a better understanding of the contribution of a seed cache to changes in body composition.

In addition to recovery of mass, Barnes *et al.* (1986, 1987) found that spermatogenesis is not advanced in the testes of golden-mantled ground squirrels (*S. lateralis*) unless the animals are euthermic. Holmes (1988) and Barnes (1984) also found that adequate energy stores are necessary for the testes to fully mature. The presence of a cache during the pre-emergence period of euthermy would allow animals to remain underground during testicular recrudescence, the cache supplying the energy both to recoup mass and to underwrite the energetic demands of euthermy and testicular growth and maturation. Indeed, Michener (1992) found that spermatogenesis was more advanced in male Richardson's ground squirrels that had spent longer periods of time euthermic prior to emergence than in males that had a short euthermic period. Thus, from the perspectives of mass recovery and sexual maturation, the advance preparation of a cache

before hibernation by males in *Spermophilus* species is probably related to the costs of reproduction in the following year (Michener 1992, 1993; Barnes *et al.* 1986). The benefits related to those costs in the previous year are recovered when the cache is consumed at the end of hibernation, immediately before the new active season, and mating, begins.

The positive correlation of size of the seed cache remaining at the end of hibernation with mass loss for animals that did survive to consume the cache suggests animals recouped more mass the more they depleted their seed caches, but this assumes that all males started with caches of similar size. The lack of correlation of the remnant cache size with either the duration of pre-emergence euthermy or the discrepancy between predicted and actual mass losses indicates no relationship between the size of the cache at the end of hibernation and the amount of seed consumed. This may also suggest that cache size at the beginning of hibernation varies between animals, an idea that is explored further in Chapter IV by examining caches of squirrels prior to the completion of hibernation.

Range in cache size in a population could be limited by several factors. First, foods must be found that are suitable for caching. The lack of caches in some male hibernacula may be explained by the lack of such discoveries or perhaps by competition for a limited supply of such items. If a suitable cache item is found, then multiple trips to collect seed are required. I occasionally trapped animals whose cheek pouches were filled with seeds, and found an average dry mass of 3.9 ± 0.9 g (range 0.8 - 7.4 g) collected by 8 animals, which means an average cache of 352 g would require in the

neighbourhood of 90 trips to prepare. Michener (1993) suggested that over 100 trips were required to prepare a barley cache she examined. These trips are energetically expensive and increase feeding demands on animals that must already achieve adequate pre-hibernation weights. Collection trips also increase the risks of conspecific aggression and predation. The benefits of collection trips would be lower if the seed is small, difficult to harvest, or has a low energetic value, so cache size likely varies depending on the ease of harvest and value of a suitable cache item. Finally, storage of the cache necessitates the expense of enlarging the hibernaculum, which likely places an upper limit on cache size.

Further study is required to determine how the factors cited in the preceding paragraph influence caching behaviours. The information gained from these studies could then be used to assess the relative benefits and costs of external vs. internal energy stores, and to evaluate what trade-offs might be made when either strategy is employed.

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Chapter IV

ARCHITECTURE AND CONTENTS OF THE HIBERNACULA OF RICHARDSON'S GROUND SQUIRRELS (*SPERMOPHILUS RICHARDSONII*)

ABSTRACT

I excavated hibernation systems of 51 Richardson's ground squirrels (*Spermophilus richardsonii*) to determine the architecture of the systems, to search for clues as to how animals had entered the hibernation system, and to expand our knowledge of seed caching by males. Hibernation systems always had a chamber (the hibernaculum), a drain tunnel or some other means of protecting the hibernaculum from flooding, and an exit tunnel. Both drain and exit tunnels varied little in diameter and did not usually branch. Drain tunnels also did not usually make abrupt changes in direction. I also often (24 of 40 systems) found 1-7 additional side tunnels that were usually shorter than the other 2 tunnel types. Males had larger hibernacula than females both in absolute terms (8.7 ± 0.6 L vs. 4.6 ± 0.4 L) and relative to body mass (16.6 ± 1.1 L/kg vs. 10.2 ± 0.8 L/kg). Males with seed caches had larger hibernacula (9.8 ± 0.6 L) than males that did not have seed caches (6.8 ± 1.0 L). No other elements of the hibernation system differed across age and sex classes. Hibernacula were found at depths of 55.8 ± 2.2 cm. Drain tunnels, which extended below the hibernaculum to depths of 82.8 ± 3.4 cm, had volumes of 3.0 ± 0.4 L. Exit tunnels, which were prepared before torpor began, were 185.1 ± 11.4 cm long and ended blindly 22.9 ± 1.9 cm below the ground surface. Squirrels excavated this remaining segment immediately before emergence in the spring. The volume of entire hibernation systems was 20.7 ± 1.84 L. I identified 5 systems that

had one or more unusual tunnels that departed from the general description of tunnel types and had unusually large volumes; I propose that these unusual tunnels are evidence of an underground connection between the hibernation system and a system used in the active season preceding hibernation.

All animals prepared a nest, usually of dry grasses, in the hibernaculum. Males lined their larger chambers with more grasses than females (256 ± 31.4 g vs. 123 ± 17.8 g dry mass). Most (23 of 35, 66 %) males but no (0 of 10) females cached seed in the hibernaculum. Caches by males weighed 292 ± 58.1 g (dry mass) and consisted of seeds of barley and sunflower that I had provided during pre-hibernation trials, barley and *Astragalus cicer* gathered from adjacent farmlands, and *Agropyron* and *Medicago*. Minor cache constituents were seeds of *Rumex*, *Alyssum*, and *Caragana*.

INTRODUCTION

Richardson's ground squirrels (*Spermophilus richardsonii*) are semi-fossorial rodents that retreat underground to burrow systems to rest or sleep, give birth and care for young, hide from predators or adverse climatic conditions, and hibernate. Richardson's ground squirrels are obligate hibernators that sequester themselves underground to hibernate for periods of 3-4 months for juvenile (< 1 year old) males, 6-7 months for juvenile females, and 8 months for adults (≥ 1 year old) of both sexes (Michener 1983). The hibernation chamber is prepared several weeks before emergence, but rarely used for sleeping before hibernation (Michener 1992, 1993, 2002). The hibernaculum is usually abandoned upon emergence, although it is sometimes used briefly (1-5 nights) as

a sleep site immediately after hibernation (Michener 1992, 2002). Furthermore, Michener (1992, 2002) reported that Richardson's ground squirrels almost never re-use the same hibernaculum in subsequent years, and they always hibernate alone.

Michener (1992, 1993) reported details of the hibernaculum chamber of Richardson's ground squirrels, but not on the remainder of the hibernation system. Reports on the nature of hibernation systems for other *Spermophilus* species have elucidated several key elements. Sexual differences in the presence of cached food and the quantity of bedding have been reported. Shaw (1925b, 1926b) found seed caches in the hibernacula of Columbian ground squirrels (*S. columbianus*), with most (92% of 13 in the latter report) having been prepared by males. He also found that adult Columbian ground squirrels tend to have more nesting material in the hibernaculum than juveniles. Young (1990), on the other hand, found no evidence of food caching in hibernacula of either male or female Columbian ground squirrels. Michener (1993, 1998) found seed caches in the hibernacula of most male but no female Richardson's ground squirrels, and she also reported that males have more nesting material than females. Male but not female arctic ground squirrels (*S. parryii*) also cache seed (McLean and Towns 1981; Buck and Barnes 1999b), as is also true of Cascade golden mantled ground squirrels (*S. saturatus*, Kenagy 1989). Criddle (1939) and Johnson (1917) reported caching behaviour in *S. tridecemlineatus*, Yahner (1978) observed that eastern chipmunks (*Tamias striatus*) changed burrows in response to available food sources in autumn when caching occurs, and Kawamichi (1989) reported food caching in the Siberian chipmunk (*Eutamias sibiricus*), but no sexual differences in this behaviour were noted for these 3 species of

sciurids.

The depth and size of hibernacula of ground squirrels often differ among age and/or sex classes within a species. Shaw (1925a, 1926a, 1926b) found that adult Columbian ground squirrels prepare larger and more elaborate hibernation systems extending to greater depths than juveniles. Young (1990), who corrected for differences in body size whereas Shaw had not, further found that adult males have larger and deeper hibernacula than adult females at a low (1470 m) elevation site, and that juveniles have the smallest and shallowest hibernacula. However, at high (2250 m) elevation, chamber size and depth did not differ significantly among the 3 age/sex classes. Michener (1992) found that volumes of hibernacula of male Richardson's ground squirrels, even adjusted for body size, were significantly larger than those of females, whereas hibernaculum depths were similar for all age and sex classes.

Soil displaced to the surface that would indicate the location of the hibernation system has not been reported. This suggests that preparation of the hibernation system by ground squirrels is accomplished by displacing the soil into an adjacent burrow system, from which the squirrel isolates itself by plugging the interconnecting tunnel(s). Shaw (1925a), in describing the escape of 2 Columbian ground squirrels into a burrow system adjacent to the hibernation system, presumed that a plugged connection had been reopened to effect these escapes. Young (1990) described a characteristic closed tunnel for Columbian ground squirrels which he presumed connected the hibernation system to another burrow system. Closed connections between active season and hibernation systems have also been found for the little ground squirrel (*S. pygmaeus*, Rall and

Demiashov 1934). The preparation of the hibernation system by extension of, and subsequent isolation from, an active season burrow system can be contrasted to the preparation of hibernation burrows by another sciurid, the Siberian chipmunk.

Kawamichi (1989) reported that most (87.8%) Siberian chipmunks selected a new system for hibernation, which was entered via a surface opening that was closed with a soil plug and, later, a mound of soil.

Hibernation systems of ground squirrels frequently include a drain tunnel. For hibernation systems of Franklin's (*S. franklinii*), northern spotted (*S. obsoletus*), and golden mantled (*S. lateralis*) ground squirrels, Wade (1930) reported tunnels that extended deeper than the hibernaculum. He assumed these provided drainage to keep the hibernaculum at least partially protected from flooding. Scheck and Fleherty (1980) likewise reported tunnels projecting below the depth of the hibernacula of thirteen-lined ground squirrels (*S. tridecemlineatus*), as did both Shaw (1925a, 1926a, 1926b) and Young (1990) for *S. columbianus*. *E. sibiricus* also prepares tunnels that extend below the level of the hibernaculum (Kawamichi 1989).

Hibernation systems are closed from the surface during hibernation, and new exit tunnels are prepared for emergence in the spring. These facts have been reported for *S. tridecemlineatus* (Johnson 1917; Scheck and Fleherty 1980), *S. columbianus* (Shaw 1925a, 1926a; Young 1990), and *E. sibiricus* (Kawamichi 1989). However, timing of preparation of the blind-ending tunnel that will serve as an exit varies among these reports. Shaw (1925a) reported that the exit tunnel was prepared before the commencement of hibernation for Columbian ground squirrels. Young (1990) found that

Columbian ground squirrels did not prepare the exit tunnel until just before emergence. The excavation of a new exit tunnel just before emergence (i.e., near the end of hibernation) has also been reported for thirteen-lined ground squirrels (Scheck and Fleherty 1980) and Siberian chipmunks (Kawamichi 1989).

Richardson's ground squirrels, like other hibernating species of *Spermophilus*, spend the majority of the year in hibernation. This fact alone warrants further investigation into the nature of systems used during hibernation, when squirrels are usually torpid and therefore vulnerable. In addition to confirming and expanding Michener's (1992, 1993, 2002) descriptions of the contents, depth, dimensions, and dedicated purpose of the hibernacula of Richardson's ground squirrels, I sought to describe the architecture and contents of the entire hibernation system to assess how Richardson's ground squirrels gain access to the hibernation system, whether drain tunnels are present, if new exit tunnels are excavated, and when the exit tunnel is prepared. Given the age and sex differences noted by Michener (1992, 1993, 2002) for some aspects of the hibernaculum and its contents, I also sought to know if any other parts of the hibernation system also differ with either age or sex of the occupant.

METHODS

Study site and Location of Hibernacula. — Richardson's ground squirrels were studied overwinter during 1997-1998 and 1998-1999 at a site located 5 km E and 1 km S of Picture Butte, Alberta, Canada (49°52'N, 112°40'W, elev. 870 m) where a colony, re-established in 1985 and 1986, has been under continuous investigation (Michener 1993,

1998, 2000). Agricultural fields border the site on 2 sides and a farmyard and gravel road border the other 2 sides, with more agricultural fields beyond. Animals were eartagged with a numbered metal tag in each ear (National Band and Tag Co., no. 1 monel self-piercing tag) on first emergence from the natal burrow, so all individuals were of known age. Fur of juveniles and older individuals was dyemarked for individual identification, and animals were regularly live-trapped (Tomahawk Live Trap Co., single-door, squirrel traps) and weighed to the nearest 5 g (Pesola AG, micro line spring scales). Mass at immergence was taken as the last mass measured before hibernation, provided that measurement was taken ≤ 10 days before immergence. Because some juvenile male squirrels lost mass in the last 4 weeks before immergence in 1998, I also report the maximum pre-hibernation mass attained by animals.

As part of a study on energy utilization by Richardson's ground squirrels during hibernation, animals were fitted during the active season with temperature-sensitive radiotransmitters (AVM Instrument Co., Models SM1-H or G3, or Austech Electronics, Model UL81T) that broadcast on unique frequencies in the range 148-149 MHz, thus enabling the identification of individual squirrels when underground. A radio receiver (AVM Model LQ-2000) and Yagi 3-element antenna were used to determine the underground location of animals by locating the point at the surface where the signal strength was strongest. Animals were located underground twice daily before, during, and after hibernation at approximately 12-h intervals, and 60-s counts of the radio signal were recorded and converted to temperature using calibration curves derived for each radio. The hibernaculum location was identified as the site where the radio signal

indicated that the squirrel had entered torpor, as determined by a drop in the radiocollar temperature to < 30 °C, and where the animal then remained for the duration of the hibernation season. The locations of 51 hibernacula were marked at the surface using spray paint and a labelled piece of lumber. I also received soil temperature data from the Lethbridge Research Centre, located 12 km south of the study site.

In an attempt to foster larger caches for a related project, I provisioned juvenile males with seed in 1997. I provided known masses of husked barley and shelled sunflower seed, both of which were dyed with food colouring and dusted with fluorescent powder, in plastic food containers (11 cm diameter, 7 cm depth) fastened to a heavy piece of linoleum (used for ballast) that was placed on the ground in areas where the subject males were known to forage. From mid-August to the first week in October, I observed for 4 1-h periods on 2 days per week while 8 juvenile males retrieved seeds on 35 occasions (number of hours of observations per squirrel varied from 1 to 9, median 6), and recorded the number of visits an animal made to the provisions and the mass of seed remaining after these visits.

For the 1997-1998 hibernation season, I located 1 adult female and 13 juvenile male Richardson's ground squirrels in their hibernacula. Two males died underground of undetermined causes, whereas the remaining 12 animals survived and emerged from hibernation. All 14 hibernation systems located in 1997-1998 were excavated. In 1998-1999, locations of hibernating squirrels were identified for 20 juvenile males, 6 juvenile females, 5 adult males, and 6 adult females. Of these 37, 26 died during hibernation; 4 adults (1 ♂, 3 ♀♀) died as a result of flooding during an intense downpour on 8 July, 4

animals (juvenile ♂♂) died of undetermined causes, and 18 were killed by a North American badger (*Taxidea taxus*; 6 juvenile ♂♂, 5 juvenile ♀♀, 4 adult ♂♂ and 3 adult ♀♀; see Michener, 2000, for a more complete account of predation on Richardson's ground squirrels by North American badgers). To assess the structure and contents of hibernation systems at the beginning of hibernation, I exhumed 1 juvenile male in its first torpor bout (4 days post-immersion) and 2 juvenile males in their second torpor bouts (10 and 11 days post-immersion). The remaining 7 juvenile males and 1 juvenile female radiocollared in 1998 survived and emerged from hibernation in 1999.

For 20 animals that survived to emerge from hibernation, hibernacula were excavated, on average, 16 ± 2.5 days after emergence (range: 1 - 37 days) and usually after I had confirmed by radiotelemetry that the owner had slept at least once in a new location. For animals killed by flooding of the hibernaculum during the heavy rainstorm of 8 July 1998, that date was assigned as the date of death. For animals killed by a badger, date of death was assigned as the date of the evening on which overnight predation occurred. For animals that died of undetermined causes, date of death was estimated as the expected midpoint of the next torpor bout after the radio failed, using one half of the duration of the previous torpor bout. This estimation could not be done for 7 animals due to the failure of their radiocollars. These 7 hibernacula were excavated only after no new emergences of squirrels were noted in the following spring and I could then safely conclude the occupants were dead. For 17 animals that died before 20 November, the delay between death and excavation was 11 ± 2.1 days (range: 0 - 25 days), whereas for 7 animals that died thereafter, when the ground was frozen, the delay

was 103 ± 15.0 days (range: 53 - 159 days). For 2 more dead animals, the decision to excavate their hibernacula was delayed 273 days each to avoid disturbing other radiocollared squirrels in hibernation nearby.

Hibernation systems were excavated using large and small hand tools. Sod, if present, was removed and then the excavation proceeded either directly down to the hibernaculum or, in the case of systems that were already opened by either badger excavation or emergence of the squirrel, from the surface exit to the hibernaculum. The surface exit opened by the squirrel was easily identifiable as a small hole dug from below with no soil displaced to the surface coincident with the observation of the newly emerged squirrel. In all cases, I confirmed that these holes were connected to the hibernaculum locations identified by radiotelemetry. Only the superior aspect of burrows and chambers was removed. All tunnel and chamber walls were explored vigorously by hand to find any loose soil that might indicate back-filled tunnels and any found were re-opened.

Once the entire system had been exposed, the contents of the chamber were removed and stored in plastic bags. A floor plan was drawn from above showing the compass orientation of the system and depicting the locations of all chambers, tunnels, and changes in horizontal direction. I inferred the location and direction of exit tunnels in some badgered systems by connecting the exit from the hibernaculum with tunnels that were transected by the badger-excavated tunnel and that did not appear to belong to any other nearby system. This allowed me to estimate the length of the exit tunnels or drain tunnels that had been partially destroyed by the badger's excavations. I made no attempt

to estimate the diameters or volumes of destroyed tunnels. I compared the length of the tunnels that I inferred with tunnels serving the same purpose in intact systems and found no statistically significant differences. I also compared the chamber contents recovered from badgered systems with those from unbadgered systems and found no differences. The data for tunnel lengths and for chamber contents were combined for subsequent analyses with no further regard for whether they were from intact or badgered systems.

System Measurements. — Depths and diameters (horizontal and vertical) of tunnels and chambers were measured to the nearest 0.5 cm. Measurements were made in the chamber and at each point where direction or depth of the tunnel made a significant change, and at all points where branching to other tunnels or termination of a tunnel was noted. Distance between points was also measured along the tunnel floor.

Compass direction (aspect) of each segment of tunnel was measured from the floor plan using a protractor. Trigonometry was used to derive x, y, and z coordinates from the depth, tunnel distance, and aspect data for each point at which measurements had been taken. The geometric centre of the chamber, derived from the horizontal and vertical diameters measured, was used as the origin of each system. These coordinate data and the diameters were entered into a modelling program (3D Studio Max, Revision 3.1, Autodesk, Inc.) to create a 3-dimensional projection and orthographic images of each system.

Volume of the hibernaculum (V_h) was calculated using the formula for an ellipsoid:

$$V_h = 4/3\pi abc$$

where a, b, and c are one half of the diameters measured in the x, y, and z directions, respectively. The minimum and maximum horizontal measurements of chamber diameter were used to derive x and y, respectively. Volume of the entire system (V_s) was calculated by adding V_h to the sum of all tunnel volumes as individually calculated by the formula for a cylinder

$$V = \pi(d/2)^2l$$

where d is the diameter of the tunnel segment and l is the length of the tunnel segment. The volume of drain tunnels, exit tunnels (the most direct route from the chamber exit to the system exit), and the volumes of all other tunnels were calculated separately.

Aspect from the centre of the chamber to the chamber exit and from the centre of the chamber to the surface exit were determined from the floor plan.

Hibernaculum Content Analysis. — I dried chamber contents to constant weight at 70°C within 12 h of removal from the hibernaculum, then re-sealed them in plastic bags until analysis. I used a set of sieves to separate dried grasses, feces, and seeds from soil or a flotation tank to separate organic material that was too fine to be separated using sieves. I used a Clipper seed cleaner (Clipper, Ltd.) to separate seed for larger samples, sieves for smaller samples, or hand-picked them when seed size precluded other methods. After re-drying to constant weight, I weighed the total amounts of dried grasses and each seed type to the nearest 0.1 g on a triple-beam balance.

Statistical Analysis. — Data on aspect from the chamber were analysed using Rayleigh's Circular test and Watson's F tests (Mardia 1972) using Oriana for Windows circular statistical software (Kovach Computing Services). Outliers were identified as

values greater than 1.5 times the interquartile range beyond the upper quartile of the distribution. Data were compared using Student's *t*-tests (paired and unpaired) and 2-way ANOVAs using age and sex as main effects (JMP-IN statistical software, version 4.0.2 for Windows, SAS Institute) on a personal computer. Post-hoc testing to determine if significant differences existed among individual age and sex classes was conducted using Tukey-Kramer tests. Significance was assumed when $P < 0.05$. All data are presented as mean \pm 1 SE.

RESULTS

Excavations. — All 14 hibernation systems located in 1997-1998 were excavated and included in analysis. In 1998-1999, 6 of 37 systems were excluded from analysis because they were either completely destroyed by a badger (1 adult σ , 2 juvenile ♀♀ , 1 adult ♀), partially collapsed as a result of flooding (1 adult σ), or were partially destroyed during excavation (1 juvenile σ). Although 14 other systems were partially damaged by badger excavation (6 juvenile $\sigma\sigma$, 3 juvenile ♀♀ , 3 adult $\sigma\sigma$, 2 adult ♀♀), the hibernaculum dimensions and contents were always recovered; however, other elements such as drain tunnels and/or exit tunnels were sometimes too damaged for assessment. Thus, of 37 candidate hibernation systems in 1998-1999, I collected at least some information from 31 (19 juvenile $\sigma\sigma$, 4 juvenile ♀♀ , 3 adult $\sigma\sigma$ and 5 adult ♀♀). Sample size varies according to the extent of destruction by badger excavation.

Of 11 adult animals radiocollared in 1998-1999, 3 males and 1 female had also been radiocollared the previous year and thus hibernation locations were known for 2

consecutive seasons. None of these 4 animals re-used the hibernaculum of the previous year. Two males (1 juvenile in 1997-1998 and 1 adult in 1998-1999) and 1 adult female (in 1998-1999) used 2 different hibernacula in the same season. The adults moved to avoid the flood of 8 July 1998, and the juvenile moved for unknown reasons, but possibly to avoid an unidentified animal that had excavated a small (2.5 cm diameter) hole at the surface which was subsequently found to be continuous with the juvenile's hibernation system. Because the second hibernaculum in each case was used out of necessity rather than by design, all 3 are excluded from all analyses.

Immergence. — In 1997, the single radiocollared adult female immersed on 28 June, and the 13 juvenile males had a mean date of immergence of 14 October \pm 3 days. In 1998, the mean date of immergence of 6 adult males was 11 June \pm 3 days, of 5 adult females was 18 July \pm 20 days, and of 6 juvenile females was 11 August \pm 3 days. For 14 juvenile males in 1998, the mean date of immergence was 23 October \pm 4 days, which was not significantly later than the same cohort in 1997 (Tukey-Kramer $P > 0.05$). However, an additional 6 juvenile males remained active in 1998 during unseasonably mild weather and delayed immergence into hibernation until 14 - 28 December (mean = 21 December \pm 2 days), 27 days or more after the immergence of the previous juvenile male and significantly later than their peers in either year (Tukey-Kramer $P < 0.05$).

At immergence into hibernation, there were no significant differences in mass between early- and late-immersing juvenile males in 1998 (431 ± 17.6 g vs. 486 ± 11.6 g), but both were significantly lighter than juvenile males in 1997 (Tukey-Kramer $P < 0.05$, Table 1). Including only the juvenile males radiocollared in 1997, juveniles had

Table 1. Immergence and maximum pre-hibernation masses of radiocollared Richardson's ground squirrels and measurements of hibernaculum systems used in 1997-1998 and 1998-1999. Data for body masses exclude juvenile males from 1998-1999 because they were significantly lighter than 1997-1998 juvenile males - all other measures were not significantly different, so data are pooled for the two years. Mass-specific data were calculated using maximum mass. Data are presented as mean \pm SE with range. Sample size is given in parentheses. Entries in the same row with different superscripts are significantly different (Tukey-Kramer). Significance was assumed at $P < 0.05$. Classes are presented in order of decreasing mass.

	Adult Males	Juvenile Males	Adult Females	Juvenile Females
Immergence body mass (g)	553 \pm 59.3 ^a 440 - 640 (3)	544 \pm 15.2 ^a 465 - 655 (13)	476 \pm 12.9 ^{ab} 430 - 510 (6)	393 \pm 21.5 ^b 340 - 430 (4)
Maximum body mass (g)	560 \pm 52.9 ^a 460 - 640 (3)	554 \pm 15.6 ^a 475 - 660 (13)	480 \pm 10.7 ^{ab} 450 - 510 (6)	418 \pm 19.8 ^b 360 - 450 (4)
Vertical diameter of hibernaculum (cm)	23.2 \pm 3.76 15.7 - 27.5 (3)	23.9 \pm 0.63 15.5 - 29.0 (31)	20.5 \pm 1.22 16.3 - 24.5 (6)	20.4 \pm 1.04 17.5 - 22.3 (4)
Minimum horizontal diameter of hibernaculum (cm)	23.8 \pm 3.42 ^{ab} 17.0 - 27.5 (3)	24.7 \pm 0.66 ^a 16.0 - 31.5 (31)	20.3 \pm 0.85 ^b 17.0 - 23.0 (6)	19.1 \pm 0.75 ^b 17.5 - 21.0 (4)
Maximum horizontal diameter of hibernaculum (cm)	25.0 \pm 3.51 ^{ab} 18.0 - 29.0 (3)	26.8 \pm 0.77 ^a 17.0 - 35.0 (31)	21.9 \pm 0.80 ^{ab} 18.5 - 24.0 (6)	20.9 \pm 1.26 ^b 18.0 - 24.0 (4)
Hibernaculum depth from surface to chamber midpoint (cm)	45.3 \pm 2.60 40.3 - 49.1 (4)	57.9 \pm 2.85 35.6 - 95.5 (31)	57.0 \pm 3.26 46.4 - 65.4 (6)	46.1 \pm 6.54 30.5 - 62.5 (4)
Tunnel diameter (cm)		8.0 \pm 0.19 6.2 - 10.0 (28)	8.0 \pm 0.33 7.3 - 8.9 (4)	7.9 \pm 0.70 6.6 - 9.9 (4)
Adjusted exit tunnel length (cm) [†]	88.0 (1)	198.9 \pm 14.37 71.5 - 337.0 (27)	146.8 \pm 14.29 94.0 - 202.5 (6)	173.8 \pm 24.72 111.0 - 226.5 (4)
Vertical distance to surface from end of exit tunnel (cm)	19.0 (1)	23.0 \pm 3.12 5.5 - 39.0 (12)	20.7 \pm 2.02 17.0 - 27.5 (5)	27.2 \pm 3.09 21.0 - 30.5 (3)

Table 1 (Continued):

	Adult Males	Juvenile Males	Adult Females	Juvenile Females
Drain tunnel length (cm)††		57.0 ± 9.32 11.5 - 169.5 (22)	41.4 ± 7.60 28.0 - 60.0 (4)	74.4 ± 14.57 51.5 - 117.0 (4)
Drain tunnel maximum depth from surface (cm)††		80.4 ± 3.61 53.0 - 115.0 (22)	82.3 ± 8.65 67.0 - 103.0 (4)	96.4 ± 14.21 68.5 - 135.0 (4)
Sum of side tunnel lengths per system (cm)†††		38.1 ± 4.66 9.5 - 83.0 (18)	53.3 ± 44.8 8.5, 98.0 (2)	36.0 ± 11.55 16.0 - 56.0 (3)
Hibernaculum volume (L)	8.1 ± 2.83 ^{ab} 2.5 - 11.5 (3)	8.7 ± 0.61 ^a 2.4 - 15.0 (31)	4.9 ± 0.59 ^b 3.0 - 6.5 (6)	4.3 ± 0.47 ^{ab} 3.6 - 5.6 (4)
Mass-specific hibernaculum volume (L/kg)	13.8 ± 4.32 5.5 - 19.8 (3)	16.9 ± 1.14 4.3 - 28.6 (31)	10.2 ± 1.27 5.9 - 14.0 (6)	10.3 ± 1.07 8.2 - 13.2 (4)
Adjusted exit tunnel volume (L)†		10.5 ± 0.86 3.4 - 18.5 (26)	8.8 ± 1.91 4.5 - 13.7 (4)	9.1 ± 1.83 3.9 - 12.5 (4)
Drain tunnel volume (L)††		3.2 ± 0.52 0.4 - 8.4 (22)	2.1 ± 0.46 1.2 - 3.2 (4)	3.2 ± 0.91 0.6 - 4.5 (4)
Sum of side tunnel volumes per system (L)†††		1.5 ± 0.18 0.3 - 3.0 (17)	0.3 (1)	1.2 ± 0.29 0.9, 1.5 (2)
Adjusted system volume - with side tunnels (L)†,††,†††		22.4 ± 3.02 7.9 - 40.3 (11)	11.6 (1)	17.5 ± 4.04 13.5, 21.6 (2)
Adjusted system volume - without side tunnels (L)†,††		21.1 ± 2.75 8.9 - 27.8 (6)	15.3 (1)	

† See methods for estimation of unexcavated exit for systems from which squirrels did not emerge. Excludes 2 unusually large exit tunnels. (See results for these and other exclusions).

†† Excludes 3 unusually large drain tunnels.

††† Excludes 6 systems in which 7 unusually large side tunnels were found.

significantly lower immergence masses than adults ($F_{1,25} = 4.87, P = 0.037$) and females had significantly lower masses than males ($F_{1,25} = 27.92, P < 0.001$). There was no significant interaction between age and sex ($F_{1,25} = 1.25, P = 0.27$). Juvenile females were significantly lighter at immergence than males of both ages, but adult males, adult females, and juvenile males did not differ significantly in immergence mass. Juvenile males radiocollared in 1998 were significantly lighter at immergence than adult males, and of similar mass to females of both ages. Because juvenile males radiocollared in 1998 were atypically small, they were excluded from pooled data (Table 1) when there were significant differences between either cohort (early- or late-immersing) and 1997 juvenile males.

I also examined the maximum mass attained by squirrels during the 4 weeks before immergence. The difference between maximum mass and immergence mass was significant for juvenile males (paired $t_{31} = 4.24, P = 0.0002$) but was not different for the other 3 age and sex classes. The difference was significant for juvenile males from 1997 (10 ± 3.4 g, $t_{12} = 2.82, P = 0.016$) and the early-immersed juvenile males in 1998 (25 ± 9.5 g, $t_{12} = 2.69, P = 0.018$); the difference for the 6 late-immersing juvenile males was not significant (5 ± 4.1 g, $t_5 = 1.22, P = 0.28$). There were no significant differences between the maximum masses of the 1997 and early-immersing 1998 juvenile males (grouped mean: 532.3 ± 12.0 g), but both were significantly heavier than the late-immersing 1998 juvenile males (436 ± 14.0 g; Tukey-Kramer $P < 0.05$).

All adult females in this study were collared for both years as part of another study (Michener 2002), so their sleep-site locations were known for every night of the active

season. Radiocollars were attached 27 ± 3.1 (range: 1 - 87) days before immergence on the remaining 44 animals; all but 1 animal had been radiocollared for at least a week before entering hibernation. Of 51 animals, only 1 was known to use the hibernaculum as a sleep site before hibernation. In 1997, a juvenile male slept for 2 nights in his future hibernaculum, slept in a previously used sleep site for 2 nights, and then returned to the hibernaculum and began hibernation. All other animals in both years were not known to sleep in the hibernaculum until the first day of sequestration. One juvenile male used a chamber that had been used in previous years by 2 adult females as a sleep site, although he was not known to sleep in that site himself during the 37 days he was radiocollared before immerging.

Emergence. — Eleven yearling males emerged in 1998 significantly earlier than 7 yearling males in 1999 (10 February \pm 4 days vs. 22 February \pm 2 days; $t_{16} = 2.34$, $P = 0.033$). The lone adult female emerged on 15 March 1999. Soil temperatures at 50 cm below the surface were 0.3°C, 0.8°C, and 2.9°C for each of those dates, respectively. The emergence mass of yearling males was not significantly different between the 2 years (grouped mean: 392 ± 11.6 g; $t_{16} = 1.80$, $P = 0.09$). The female's emergence mass was 223 g.

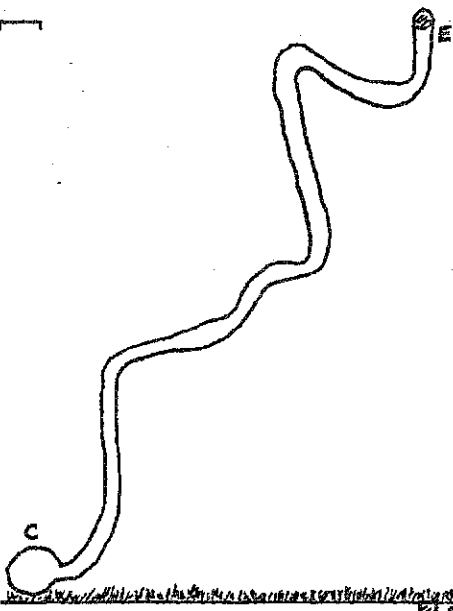
Use of the hibernaculum as a sleep site after emergence was limited to 6 of 16 (37.5%) animals for which data were available. Ten animals slept in a new location on the first night after emergence. Two animals used the hibernaculum as a sleep site for 9 and 10 nights before radiocollars were removed. The remaining 4 animals used the hibernaculum as a sleep site for 2 ($n = 2$) and 3 ($n = 2$) nights before using another sleep

site.

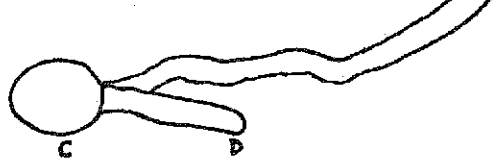
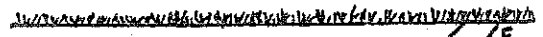
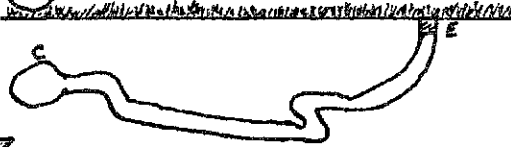
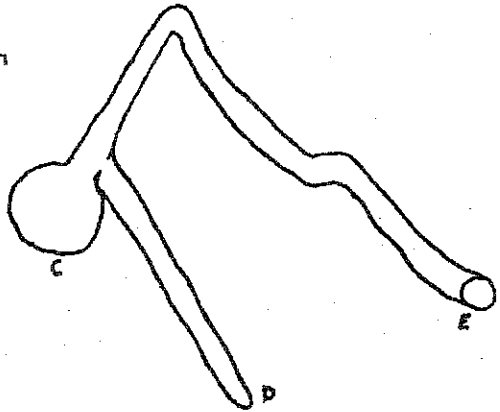
Architecture of Hibernaculum Systems. — Richardson's ground squirrels construct a relatively simple hibernaculum system. In every case, the squirrel was isolated from the surface, and the systems were prepared without ever, to my knowledge, removing soil to the surface. Every system consisted of an ellipsoidal chamber connected to an exit tunnel that rose near to the surface but did not open at the surface unless the animal had emerged from hibernation. The exit tunnel in systems not vacated by the occupant stopped at undisturbed soil, as evidenced by the presence of roots and rhizomes from surface plants, confirming that this was an end to excavation and not a soil plug. At the junction between the chamber and the exit tunnel, there was a small but definite rise from the tunnel floor before dropping into the chamber that I call the hibernaculum lip. Another tunnel, usually connected to the exit tunnel, close to its juncture with the hibernaculum, was found in 34 of 40 (85%) systems. Because this tunnel descended to a blind end deeper than the hibernaculum, I called it a drain tunnel. The deepest point in all drains coincided with the blind end of the tunnel, and that point was reached by a tunnel with only minor variations in diameter and which never branched. In 6 systems, no drain tunnel was found. However, in 1 of these 6 the extraordinarily long exit tunnel (531.5 cm) ran at a greater depth than the hibernaculum lip (54 cm) for 415.5 cm (78% of the total length; Fig. 1-1), at one point reaching a depth of 80 cm below the surface or 26 cm below the lip. In the remaining 5 systems, the hibernaculum was the lowest point in the system. In a majority (24 of 40, 60%) of systems, additional side tunnels were found. These side tunnels usually arose from the exit tunnel but occasionally from the chamber

Figure 1. Orthographic renderings of 6 hibernation systems of Richardson's ground squirrels representing one unique organization (1) and examples of the 3 basic plans (2-6). Each drawing includes a view of the entire system from above (top) and from the side (below), with the ground surface shown. 1: An unusual system with no side or drain tunnels, but with an exit tunnel that is below the level of the hibernaculum for 4.2 m of its 6.1-m length; 2 and 3: the exit tunnel connects directly to the chamber with a drain tunnel branching to the side; 4 and 5: the exit tunnel connects directly to the drain tunnel with the chamber arising from a branch to the side; 6: similar to 2 and 3 except that the drain branches from a dip in the exit tunnel. C = Hibernaculum Chamber; D = Drain Tunnel; E = Exit (to surface). Diagonal shading indicates proposed (shortest) exit routes that were not excavated by squirrels that had not completed hibernation. These systems illustrate the isolation of the occupant from the surface or any other underground systems. Scale indicated on each drawing is 20 cm.

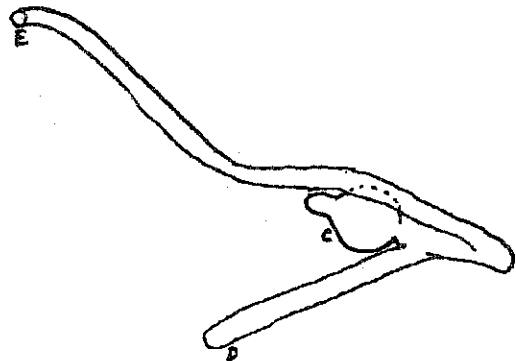
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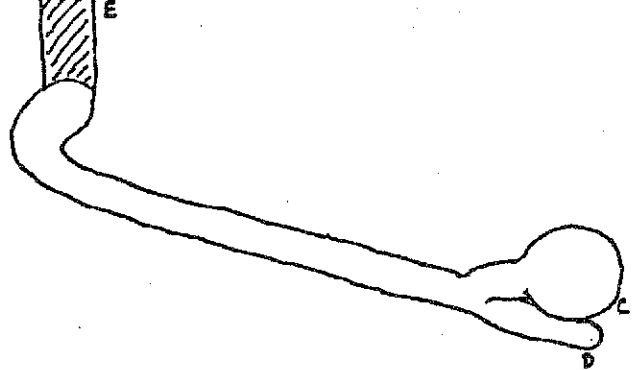
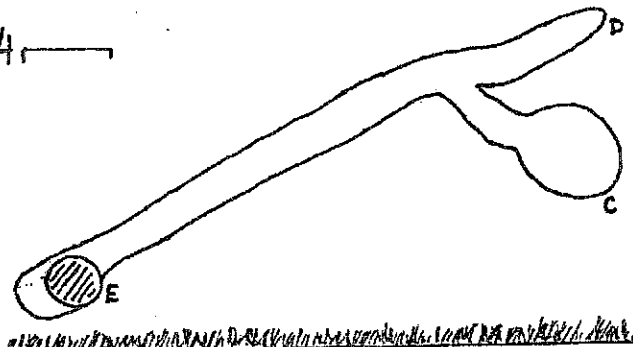
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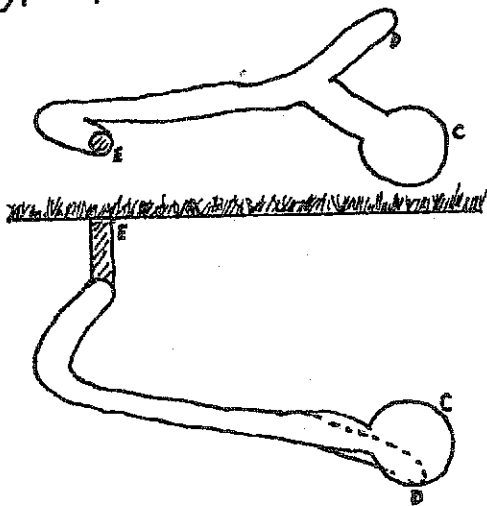
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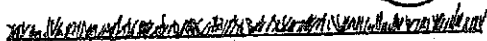
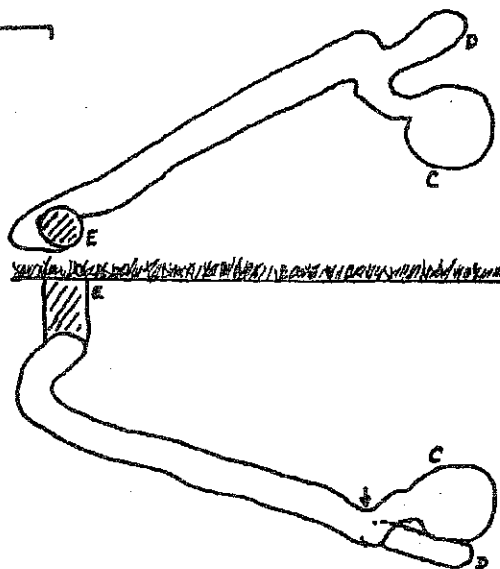
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itself, in which case the side tunnel was always directly opposite the opening from the chamber to the exit tunnel. The presence of a single side tunnel was the most frequent configuration ($n = 13$), but there were also systems with 2 ($n = 3$), 3 ($n = 4$), 4 ($n = 2$), 5 ($n = 1$), and 7 ($n = 1$) side tunnels. There were no side tunnels in 16 systems. Side tunnels were shorter than most drain and exit tunnels, always ended blindly, tended to run nearly level and straight from their point of origin, and had only minor variations in tunnel diameter.

Within the basic design (a chamber, an exit tunnel, a drain tunnel, and perhaps 1 or more additional side tunnels), 3 main plans were identified to describe how the drain tunnel related to the exit tunnel. The most common plan ($n = 15$) was an exit tunnel that ran directly into the chamber and a drain tunnel that arose as a branch from the exit tunnel (Figs. 1-2, 1-3). The entrance to the drain in this plan was at the same level as the exit tunnel to either side. In the second plan ($n = 9$), the exit tunnel ran directly into the drain and the chamber arose as a branch to one side (Figs. 1-4, 1-5). The third plan ($n = 8$) featured a modification of the first plan. The exit tunnel ran directly to the chamber, but the drain tunnel arose from a dip in the exit tunnel such that the entrance to the drain was lower than the exit tunnel to either side (Fig. 1-6).

Connections to Other Systems. — I used the measurements and descriptions of the majority of drain or side tunnels (length, volume, presence of enlarged areas, changes in elevation or direction) as 5 criteria to identify unusual tunnels. Systems that had tunnels which were unusual in only 1 or 2 respects when compared to tunnels in the majority of systems, are reported simply as outliers. Ten systems (2 adult ♀♀ and 8 juvenile ♂♂)

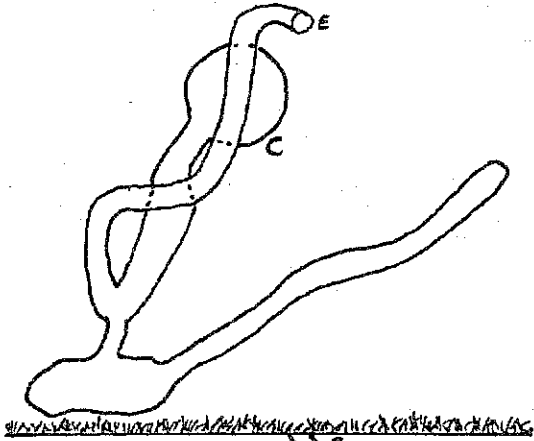
were distinctly different from the remaining 35 systems, with unusual tunnels that violated at least 3 of the 5 criteria. In each case, I found that volumes of certain tunnel constituents were considerably larger than the same constituents of the majority of systems. In 3 systems, all belonging to juvenile ♂♂, tunnels that I had originally designated as drains had volumes of 12.6, 13.8, and 22.0 L compared with the mean for 30 other systems of 3.0 ± 0.40 L. The enlarged volumes in all 3 were due both to having somewhat longer lengths and larger diameters of the drain tunnels, though neither length nor diameter for any of the 3 were outliers. In another 5 systems (3 juvenile ♂♂ and 2 adult ♀♀), the summed side-tunnel volumes were 8.3, 12.0, 19.5, 20.3 and 28.1 L, whereas the mean for 23 other systems was 2.1 ± 0.42 L. Exit tunnel volumes in 2 other systems (juvenile ♂♂) were 24.3 and 32.9 L, whereas the mean for 35 other systems was 9.5 ± 0.71 L.

I re-examined the 3-dimensional drawings of each system and concluded that 5 of the 10 systems with unusual tunnels provided evidence of a connection between the hibernation system and at least a part of another system. The 3 unusually large drains departed from the general description of drain tunnels given above. Each had a region in which the tunnel diameter was much larger than other parts of the tunnel, and each tunnel was either branched or made abrupt changes in direction at the junction with the enlarged area (Figs. 2-1, 2-2, 2-3). These 3 drain tunnels were excluded from further analyses.

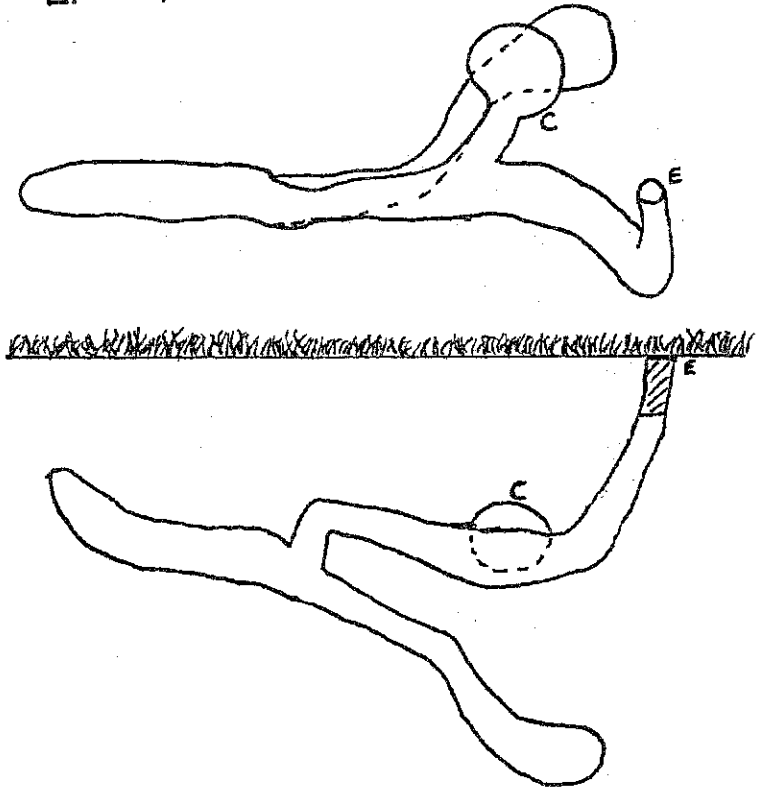
Of the 5 systems with large side-tunnel volumes, the 2 with lower volumes were otherwise similar to basic systems. The 1 with the lowest side-tunnel volume had 4 side tunnels that were not in themselves unusual but merely increased the total side-tunnel

Figure 2. Orthographic renderings of 5 unusual hibernation systems of Richardson's ground squirrels. Each drawing includes a view of the entire system from above (top) and from the side (below), with the ground surface shown. Scale (20 cm) is indicated on each drawing. Tunnels connected to the exit tunnel with unusually large volumes (stippled) that have enlargements, branches, or marked changes in elevation and/or direction are proposed to be elements of active-season systems from which the hibernation system was accessed. Diagonal shading indicates proposed (shortest) exit routes that were not excavated by squirrels that had not completed hibernation.

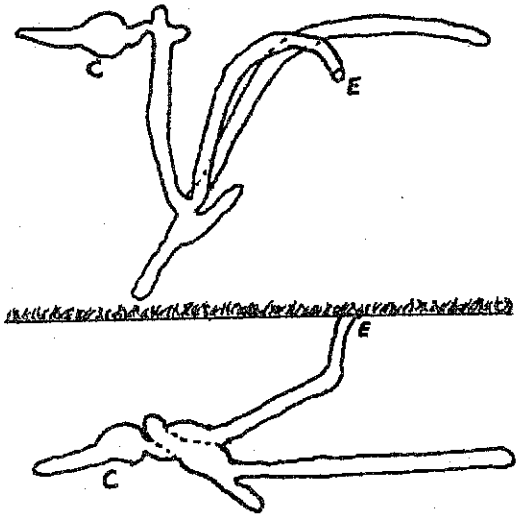
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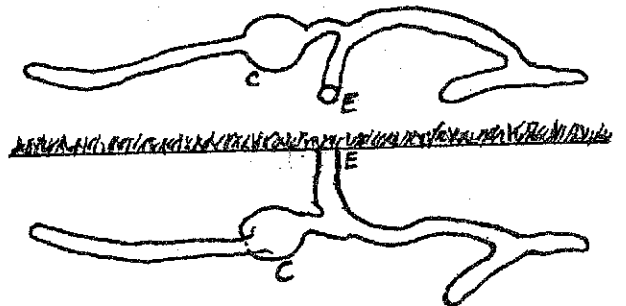
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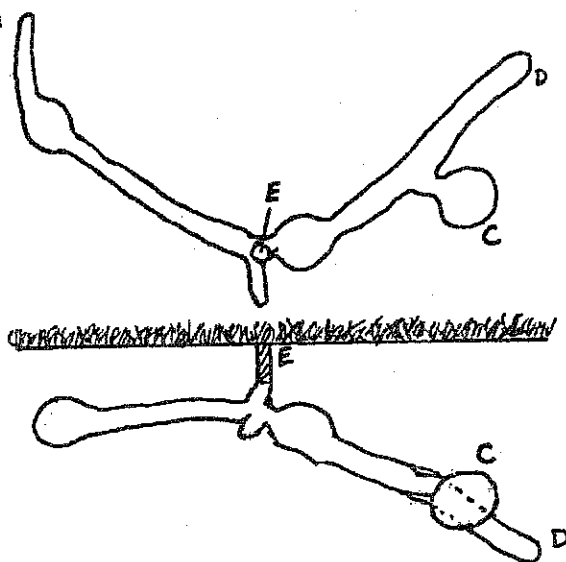
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volume. The second had a long side tunnel with a bifurcation at the end, but did not otherwise depart from the description of other side tunnels. I decided these 2, belonging to juvenile ♂♂, did not necessarily provide evidence of connections to other systems based on their modestly larger volumes alone, and so report them as outliers in the following analyses.

The remaining 3 systems had 1 side tunnel that was different from other side tunnels. One of these larger side tunnels had substantial amounts of loose soil throughout and was connected to a plugged but previously open exit to the surface. I concluded that this system had been invaded by another squirrel after the hibernating occupant (an adult ♀) had died in the flood of 8 July 1998. The partially consumed carcass of the dead female was found at the bottom of this side tunnel. Thus, although it appeared to be evidence of a connection between a hibernation system and another system, the 2 systems probably belonged to 2 different animals, or at least 1 had been modified by another animal. A second unusual side tunnel branched once, had regions with considerably larger diameters than other parts of the same tunnel, and varied in its elevation more than other side tunnels (Fig. 2-4). The occupant (another adult ♀) somehow escaped this hibernation system without exiting to the surface, probably in response to the flood of 8 July 1998. She did not emerge in the following spring, and was presumed to have died underground, but I did not find her body. There was no evidence (such as loose soil) that the system had been invaded as there was in the previous example. The third unusual side tunnel was part of a system with 2 additional chambers. One of these extra chambers existed between the hibernaculum (confirmed as such both by its location relative to the

location determined by radiotelemetry and the fact that it was the only 1 with any bedding) and the exit. The side tunnel of this system was particularly long, curved, and had a second extra chamber (Fig. 2-5). Based on these departures from the usual plan, I concluded that these latter 2 side tunnels were also evidence of a connection from the hibernation system to another system. The unusually large side tunnels in these latter 3 systems were excluded from further analyses.

The exit tunnels in the 2 systems with larger than usual exit-tunnel volumes were remarkably long and therefore the exit-tunnel volumes were much greater than those of other systems. However, neither tunnel was branched nor enlarged at any point, nor were they notable in any other respect and so they are reported as outliers in the following analyses.

Dimensions of Hibernaculum Systems. — Despite the differences found between immergence and maximum masses of juvenile $\sigma\sigma$ in 1997-1998 and early- and late-immerging juvenile $\sigma\sigma$ in 1998-1999, none of the measurements of hibernation systems were significantly different between those 3 groups, so all data for juvenile $\sigma\sigma$ are pooled. Vertical diameter, available for 43 hibernacula of all age and sex classes, was significantly smaller than the minimum horizontal diameter, which was in turn significantly smaller than the maximum horizontal diameter (paired $t_{42} \geq 2.15$, $P \leq 0.038$ for all 3 pairwise comparisons). Vertical diameters were 0.6 ± 0.29 cm (2.3 %) smaller than minimum horizontal diameters, which were 1.9 ± 0.19 cm (7.3%) smaller than maximum diameters. None of these differences varied significantly among age and sex classes ($F_{1,42} \leq 1.77$, $P \geq 0.18$ for all 3 pairwise comparisons). In other words, the cross-

sections of hibernacula varied from circular in all 3 planes (x-y, x-z, and y-z) equally for all 4 age and sex classes (Table 1). One unusual chamber, belonging to a juvenile male, had a vertical diameter 4.8 cm larger than the minimum horizontal diameter and 4.3 cm larger than the maximum horizontal diameter.

Mean diameters of chambers for 34 males were greater than mean diameters for those of 10 females ($F_{1,42} \geq 4.55$, $P \leq 0.039$), but there were no age-related differences ($F_{1,42} \leq 0.06$, $P \geq 0.80$). V_h of males was significantly greater than that of females (34 males: 8.7 ± 0.60 L; 10 females: 4.6 ± 0.40 L; $F_{1,42} = 8.87$, $P = 0.005$) whereas there were no significant age-related differences ($F \leq 0.01$, $P \geq 0.98$). Hibernacula of juvenile males were significantly larger than those of adult females, but there were no other significant differences among age and sex classes in V_h . One adult male's V_h was less than a quarter that of the other 2 adult males (2.5 vs. 10.4 and 11.5 L); 2 juvenile males also had similarly small V_h 's (2.4 and 2.8 L). This variability and the small sample size for adult males meant that there were no significant differences when V_h for adult males was compared to those of either adult or juvenile females. Adjusting for the size of the occupant by calculating the mass-specific V_h (by dividing the volume by the maximum mass attained by the occupant in the 4 weeks before immergence) did not abolish the sex-difference: mass-specific V_h was significantly greater for males than females ($F_{1,42} = 5.00$, $P = 0.031$). There were no age-related differences in mass-specific V_h . The 1 adult male with the small V_h had a lower maximum mass than the other 2 adult males and yet still had a much smaller mass-specific V_h (5.5 vs. 16.3 and 19.8 L/kg). Males ($n = 34$) had significantly larger hibernation chambers than females ($n = 10$) even when adjusted for

body mass (16.6 ± 1.10 L/kg vs. 10.2 ± 0.83 L/kg). Males that had seed caches ($n = 21$) had significantly larger chambers (9.8 ± 0.6 L) than males that did not have seed caches ($n = 13$; 6.8 ± 1.0 L; $t_{32} = 2.675$, $P = 0.012$). Maximum pre-hibernation body mass of squirrels with seed caches did not differ from that of squirrels without seed caches ($t_{32} = 0.056$, $P = 0.96$), and so the mass-specific V_h was also significantly larger for caching males (18.8 ± 1.08 L/kg) than for non-caching males (13.1 ± 1.97 L/kg; $t_{32} = 2.742$, $P = 0.010$). Both V_h and mass-specific V_h were significantly positively correlated with the size of the seed cache (V_h : $r^2 = 0.22$, $P = 0.006$; mass-specific V_h : $r^2 = 0.23$, $P = 0.005$). Depths of hibernacula at the vertical midpoint of the chamber did not differ significantly among age and sex classes ($F_{1,42} = 0.32$, $P = 0.73$). Mean depth was 55.8 ± 2.21 cm ($n = 44$, range: 30.5 - 95.5 cm). Neither V_h nor mass-specific V_h were correlated with depth of the hibernaculum (V_h : $r^2 = 0.04$, $F_{1,42} = 1.56$, $P = 0.22$; mass-specific V_h : $r^2 = 0.03$, $F_{1,42} = 1.38$, $P = 0.25$).

The mean diameters of all tunnels were similar whether they were exit, drain, or side tunnels (paired $t_{18} \leq 1.25$, $P \geq 0.227$ for all 3 pairwise comparisons). Tunnel diameters averaged 8.0 ± 0.15 cm in 37 systems (range: 3.5 - 11.8 cm), and these did not differ significantly across age and sex classes ($F_{1,35} = 1.276$, $P = 0.29$). The diameter of the exit hole excavated by 17 emerged squirrels was significantly smaller than the mean tunnel diameters of the same systems (paired $t_{16} = 5.18$, $P < 0.001$). Exit hole diameters were 6.1 ± 0.25 cm (range: 4.5 - 8.25 cm), were not correlated with tunnel diameter ($r^2 = 0.05$, $F = 2.06$, $P = 0.37$), and did not vary significantly among age and sex classes ($F_{1,15} = 2.84$, $P = 0.11$).

Exit tunnel length was significantly shorter for animals that had not emerged ($n = 21$; 146.1 ± 12.01 cm) when compared to those that had emerged ($n = 17$; 205.0 ± 20.82 cm; $t_{36} = 2.56$, $P = 0.015$) because the opening to the surface was not excavated in the former. Exit tunnel volumes, though smaller for closed systems ($n = 18$; 9.1 ± 0.91 L) than for open systems ($n = 17$; 10.0 ± 1.12 L), were not significantly different ($t_{33} = 0.58$, $P = 0.57$). I added the vertical (shortest) distance between the end of the exit tunnel and the surface to the exit tunnel length of closed systems to create an adjusted exit tunnel length that approximated the anticipated length of the exit tunnel had squirrels emerged. I used the mean diameter of tunnels in each system to calculate the equivalent tunnel volume that this approximation would add to exit tunnel volume, and therefore V_s . The mean vertical distance remaining to be excavated in closed systems was 22.9 ± 1.90 cm ($n = 21$; range: 5.5 - 39.0 cm), with an estimated volume of 1.3 ± 0.14 L ($n = 18$). In 11 systems from which squirrels had emerged, a steep rise occurred in the exit tunnel at 18.1 ± 2.62 cm below the surface (range: 12.0 - 43.0). I compared this depth to that of the depth of the terminal end of closed systems and found no significant difference ($t_{31} = 1.35$, $P = 0.19$). The vertical distances remaining to be excavated at the time of emergence at the end of the exit tunnel in closed systems did not differ significantly among age and sex classes ($F_{1,19} = 0.56$, $P = 0.58$), nor did the estimated volumes ($F_{1,17} = 0.30$, $P = 0.74$). The mean adjusted exit-tunnel length was 169.0 ± 0.11 cm ($n = 21$), and the difference between open and adjusted exit-tunnel lengths was no longer significant ($t_{36} = 1.61$, $P = 0.12$). I therefore combined open and adjusted measurements for all further analyses. Exit-tunnel length was not statistically significantly different among age

and sex classes ($F_{1,32} = 1.09$, $P = 0.35$), nor were exit-tunnel volumes ($F_{1,32} = 0.41$, $P = 0.67$). Of 40 hibernation systems, 2 had extraordinarily long exit tunnels of 545.5 and 612.5 cm. Neither of these systems was located in an area where the soil was more easily removed by me than for other systems, nor were there any obstacles that could explain the extraordinary length. The other 38 systems had exit tunnels that averaged 185.1 ± 11.39 cm (range: 71.5 - 337.0 cm) in length. Exit-tunnel volumes averaged 10.2 ± 0.72 L ($n = 35$; range: 3.4 - 18.5 L). This mean excludes the 2 longest exit tunnels, which had volumes of 24.9 L and 32.9 L, respectively.

Excluding the 3 unusually large drain tunnels already mentioned, drain tunnel length, volume, and depth did not differ significantly among age and sex classes ($F_{1,29} < 1.30$, $P > 0.281$ for all 3). The 30 drain tunnels were 57.2 ± 7.24 cm long (range: 11.5 - 169.5 cm), had an average volume of 3.0 ± 0.40 L (range: 0.4 - 8.4 L), and reached an average depth of 82.8 ± 3.42 cm (range: 53.0 - 135.0 cm).

Aside from the side tunnels in systems that I concluded were connected to active season systems, I found 44 side tunnels in 23 hibernation systems (19 juvenile ♂♂, 1 adult ♀, 3 juvenile ♀♀). The destruction of the systems of adult males by a badger precluded finding side tunnels for this class. Seven of the 44 (16%) side tunnels, found in 5 systems, were unusually large with average lengths of 82.9 ± 14.40 cm (range: 52.0 - 154.0 cm) and average volumes of 4.2 ± 0.45 L (range: 2.6 - 5.8 L). The majority (37 of 44, 84%) of side tunnels averaged 21.7 ± 1.61 cm in length (range: 6.5 - 43.5 cm) and 0.9 ± 0.09 L in volume (range: 0.2 - 2.1 L). Lengths of all 44 side tunnels did not differ significantly among any of the 3 classes that did have side tunnels ($F_{1,42} = 0.36$, $P = 0.70$),

nor did side tunnel volumes ($F_{1,42} = 0.36$, $P = 0.70$). However, small sample sizes for females of both ages renders this finding inconclusive. Ten normal tunnels were found in 4 systems which also included 1 ($n = 2$) or 2 ($n = 2$) of the unusual tunnels (the seventh unusual side tunnel was the sole side tunnel in the fifth system). The 5 systems with unusual side tunnels were excluded from further analyses. The 27 remaining side tunnels contributed to a mean total side tunnel length of 31.7 ± 4.03 cm (range: 8.5 - 66.3 cm) per system in 18 systems and accounted for a mean total volume of 1.4 ± 0.18 L (range: 0.3 - 3.0 L) per system.

V_s was calculated for 21 systems (17 juvenile $\sigma\sigma$, 1 adult φ , 2 juvenile $\varphi\varphi$) that did not have unusual exit tunnels, drain tunnels, and/or side tunnels. V_s of surviving ($n = 12$) and adjusted V_s of non-surviving squirrels ($n = 9$) did not differ significantly ($t_{19} = 0.42$, $P = 0.68$). When V_s for open systems was pooled with adjusted V_s for closed systems, there were no significant differences in V_s among age and sex classes ($F_{1,19} = 1.08$, $P = 0.36$). V_s for these 21 animals was 20.7 ± 1.84 L (range: 7.9 - 40.3 L). The mean V_s for animals with side tunnels ($n = 14$) was 20.9 ± 2.53 (range: 7.9 - 40.3) whereas for animals that did not have side tunnels ($n = 7$) V_s was 20.3 ± 2.47 (range: 8.9 - 27.8). These means were not significantly different ($t_{19} = 0.15$, $P = 0.88$).

The direction of the exit from the chamber from its centre was not significantly different from a random distribution ($R = 0.18$, $P = 0.24$), but the location of the exit hole to the surface relative to the chamber location was concentrated in a north-northeast direction. This distribution was significantly non-random (mean direction: 18.4° ; $R = 0.29$, $P = 0.027$, Fig. 3).

Hibernaculum contents. — Most (43 of 45, 96%) squirrels filled their hibernacula exclusively with dried grasses. However, the hibernacula of 2 juvenile males located 3 and 20 m from a vacated farm building contained 65 g and 536 g of pink fibreglass insulation. The chamber of the former animal also included 169 g of grasses, whereas that of the latter contained almost exclusively fibreglass, with just 10 g of grasses. These 2 animals were excluded from further analyses of bedding. Juvenile males had a greater mass of dry grasses in the hibernaculum than any other age and sex class, but this difference was not statistically significant ($F_{1,40} = 3.05$, $P = 0.06$; Table 2). However, males ($n = 33$) had a significantly greater mass of grasses than females (males: $n = 33$, 256 ± 31.4 g; females: $n = 10$, 123 ± 17.8 g; $t_{41} = 2.273$, $P = 0.028$). As this difference mirrored the difference in the body masses, I adjusted for body mass by dividing the dry mass of the bedding by the maximum pre-immersion mass of the animal that used the nest. The difference in mass-specific dry mass of grass bedding between the sexes was not significant (males: $n = 33$, 493 ± 61.0 g/kg; females: $n = 10$, 273 ± 42 g/kg; $t_{41} = 1.92$, $P = 0.06$), nor were there any significant differences among the 4 age and sex classes ($F_{1,41} = 2.43$, $P = 0.10$). Of the 3 hibernacula from which I excavated torpid animals shortly after immersion, I observed 2 of the animals before they began arousing appreciably; the third had aroused enough in response to my excavation by the time I changed its body position to do so. The other 2 animals were sitting in a ball, the caudal dorsum downward and the rostral dorsum uppermost in the nest, the body curled ventrally, the head tucked under the tail, and the nose apposed to the abdomen. Each animal was surrounded on all sides by the grasses that filled the nest. The only opening

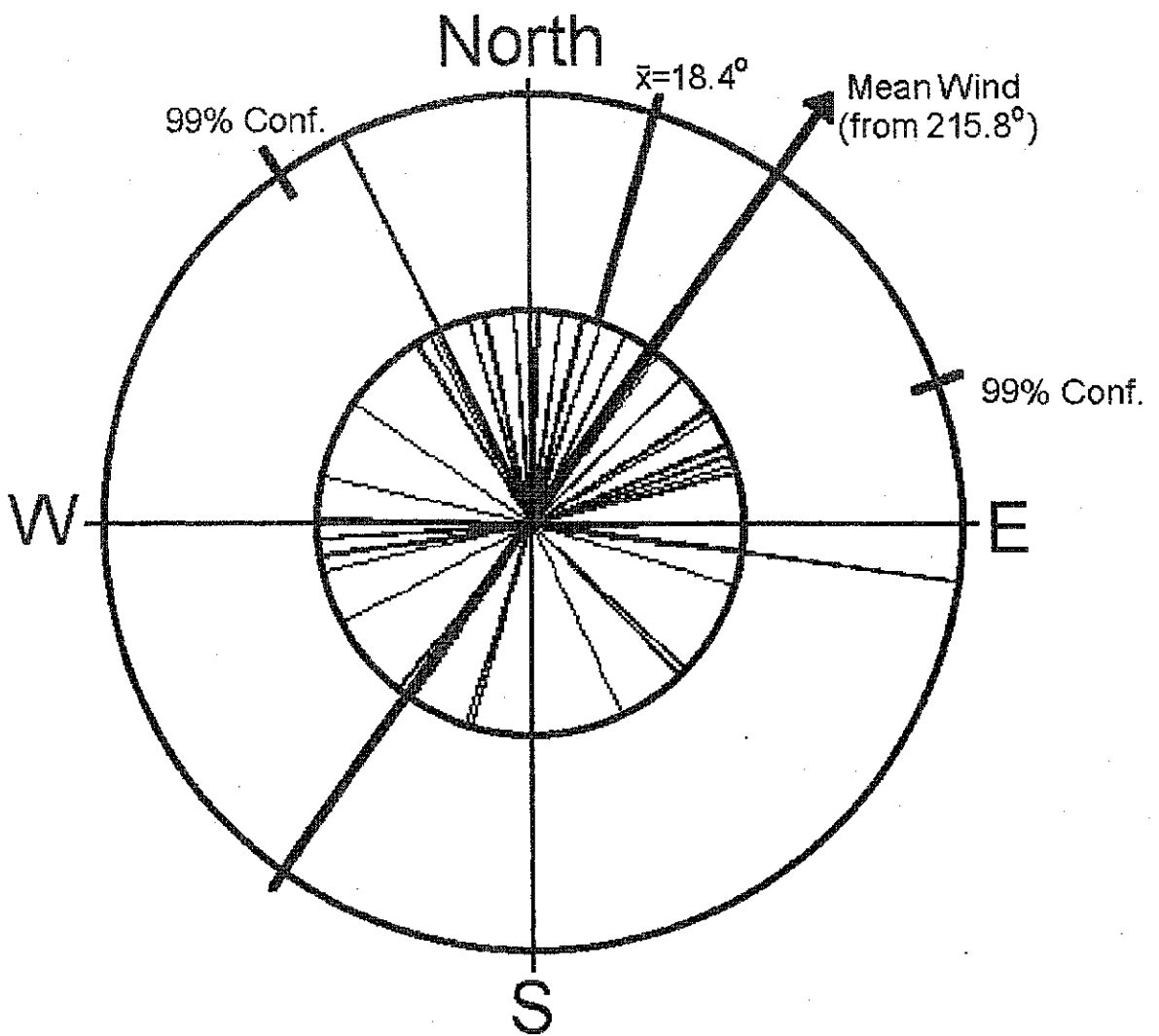


Figure 3. Directions of the exit tunnels of 43 hibernacula of Richardson's ground squirrels. Each line radiating from the centre represents the direction of a single exit tunnel from the chamber to the end of the exit tunnel. Double length rays indicate 2 tunnels had the same direction. Mean direction of all of the tunnels was 18.4° , which was significantly different from a random distribution ($R = 0.29, P = 0.027$). 99% confidence intervals for the mean tunnel direction are indicated.

Table 2. Contents of hibernacula of Richardson's ground squirrels pooled for 1997-1998 and 1998-1999. Mass-specific data were calculated using maximum pre-immersion mass. Data are presented as mean \pm SE with range. No age-related differences were found (ANOVA, $P > 0.05$) but males had more bedding than females, and were the only ones to hibernate with seed caches. Mass-specific masses of bedding did not differ between males and females. Sample size (shown in parentheses) for bedding excludes 2 juvenile males that collected pink fibreglass for nesting material.

	Adult Males	Juvenile Males	Adult Females	Juvenile Females
Dry mass of bedding (g)	197 \pm 74.7 35 - 386 (4)	264 \pm 34.4 42 - 686 (29)	119 \pm 10.8 91 - 160 (6)	130 \pm 45.4 78 - 266 (4)
Mass-specific dry mass of bedding (g/kg)	323 \pm 115.6 77 - 607 (4)	512 \pm 67.4 80 - 1333 (29)	249 \pm 23.9 182 - 319 (6)	310 \pm 105.4 187 - 626 (4)
Dry mass of cached seed (g)	220 \pm 123.7 3 - 431 (3)	333 \pm 68.4 3 - 914 (20)	0 (6)	0 (4)

in the grasses, other than the one made by me to retrieve the torpid animal, was a small sphere in roughly the centre of the entire mass of grasses (and therefore the centre of the chamber) where the animal was found.

Most males (23 of 35, 66%) cached seed, whereas none of 10 females cached seed. The seed was usually recovered from the lower part of the hibernaculum with dried grasses both above and below the seed, so that it was effectively isolated both from the soil floor of the chamber and from direct contact with the animal. When more than 1 seed type was present, discrete piles of each seed type could often be identified, though this separation was not perfect and some mixing of seed types did occur. Seed was found in the hibernacula of 21 of 31 (68%) juvenile males and 3 of 4 (75%) adult males. Nine of 13 (69%) and 12 of 18 (67%) juvenile males hibernated with seed caches in 1997-1998 and 1998-1999 respectively. The mass of seed caches of juvenile and adult males did not differ significantly ($t_{21} = 0.21$, $P = 0.65$; Table 2). Average dry mass of 23 seed caches was 292 ± 58.1 g (range: 3 - 914 g). Four seed caches weighed <5 g and 6 others weighed 21 - 124 g. The remaining 13 caches were evenly distributed from 209 to 914 g. Of those males with cached seed, mean cache size for emerged squirrels ($n = 14$, 342 ± 81.4 g, range: 3 - 914 g) was greater than for squirrels that died in hibernation ($n = 8$, 171 ± 71.0 g, range: 3 - 511) but this difference was not significant ($t_{20} = 1.42$, $P = 0.17$). Only one of the 3 juvenile males excavated early in hibernation had a seed cache, which was 547 g. The size of the seed cache for males that survived hibernation was not correlated with any of time spent euthermic either after immergence or before emergence, total time euthermic, or time in hibernation, nor was it correlated with any of the rates of

mass loss calculated per euthermic day after immergence, per total euthermic days, or per days in hibernation ($r^2 \leq 0.20$, $F \leq 2.29$, $P \geq 0.16$ for all comparisons). However, cache size was positively correlated with the amount of mass lost during hibernation ($n = 14$, $r^2 = 0.41$, $F = 8.36$, $P = 0.014$) and, for a subset of the same males for which radiotelemetric records were complete, the size of the remnant seed cache was also positively correlated with the rate of mass loss per day euthermic before emergence ($n = 11$, $r^2 = 0.43$, $F = 6.81$, $P = 0.028$).

Of the 2 types of provisioned seed from the trials conducted in 1997, I recovered dried masses of 584 and 1 g of marked barley from 2 hibernacula. These 2 animals had been observed collecting a total of 220 and 14 g of marked barley, respectively. The first animal had been observed to collect less barley than was found in his hibernaculum. The second animal had also collected 9 g of sunflower seed, of which none was found in his hibernaculum. Marked sunflower seed was also recovered from the first hibernaculum (171 g) and from a third hibernaculum (25 g). The occupant of the first hibernaculum had been observed to collect only 88 g of sunflower. The third hibernaculum was used in 1998-1999 by a juvenile that had not yet been born when I conducted the provisioning in 1997. Three other animals had collected 221 ± 50.6 g (range: 134 - 309 g) of sunflower seed, and these 3 animals and 1 other had collected 219 ± 88.1 g (range: 18 - 440 g) of marked barley, but none of this provisioned seed was found in any of their hibernacula. Based on the mismatches between which animals had been observed to collect seed and which had seed caches in the hibernaculum, and between the masses collected and those found in caches, I conclude that animal activity after the time of seed collection results in

the distribution of seed in an unpredictable fashion. Unlike any other seed that I recovered from caches, the marked seed was mouldy in every instance, and thus of dubious food value. All other (i.e. not provided by me) seed was very dry and no signs of deterioration or decay were noted. Unmarked barley, presumably collected from nearby agricultural fields by squirrels, was found in another 3 hibernacula. In total, barley was the only seed type in 1 cache, the major constituent (>50% of dry mass of cache) in 3 others, and a minor constituent ($\leq 50\%$) in 1 other cache (Table 3). In the 5 caches in which it was found, barley contributed 317 ± 112.7 g, the highest average of all seed types and larger than the mean cache size of 292 g. The smallest cache of marked barley (1.3 g) was complemented by an equal amount of *Astragalus cicer* (cicer milkvetch), forming 1 of the 4 smallest seed caches recovered. *A. cicer* formed the sole constituent in 3, the major constituent in 5, and a minor constituent in 5 caches. On average, milkvetch contributed 198 ± 68.5 g to 13 caches. Seeds of *Agropyron* sp. (wheatgrass) were found in 13 caches, and were the sole constituents in 1, the major constituents in 4, and the minor constituents in 8 caches. *Agropyron* caches averaged 143 ± 43.6 g. *Medicago lupulina* (black medick) was the fourth most abundant cached seed type. From 13 caches, an average of 31 ± 10.5 g of *Medicago* seed was recovered, and it was the sole constituent in 1 small (2.5 g) cache, the major constituent in 2 caches (total cache: 5 and 56 g), and a minor constituent in 10 other caches (range: 21 - 914 g). Other minor constituents of caches were *Rumex* sp. (sorrel: 73 g in 1 cache), *Alyssum alyssoides* (peppergrass: 46 g in 1 cache), and *Caragana arborescens* (3 and 17 g in 2 caches). Of 23 caches recovered, 6 were composed of only 1 seed type, 8 were mixtures of 2 types, 6 had 3

Table 3. Constituent seeds in hibernaculum caches of 23 male Richardson's ground squirrels in 1997-1998 and 1998-1999. Data are presented as mean \pm SE (range). Mean dry masses of 100 seeds were based on the mass of 300 seeds. Major constituent column excludes caches in which the seed was the sole constituent. Bar - Barley; Agr - *Agropyron*; Ast - *Astragalus*; Med - *Medicago*; Sun - Sunflower; Rum - *Rumex*; Aly - *Alyssum*; Car - *Caragana*. Some barley and all sunflower seeds were provided by the author.

Seed type	Mean dry mass of 100 seeds (g)	Total caches (n)	Mean dry mass per cache (g)	Sole constituent (n)	Major constituent (n)	Minor constituent (n)	Number of caches with other seed species included								
							Bar	Agr	Ast	Med	Sun	Rum	Aly	Car	
Monocots															
Barley	1.72	5	317 \pm 112.7 (1.3 - 583.9)	1	3	1	-	2	2	2	1	0	0	2	
<i>Agropyron</i>	0.66	13	143 \pm 43.6 (0.1 - 430.4)	1	4	8	2	-	6	8	1	1	1	1	
Dicots															
<i>Astragalus</i>	0.36	13	198 \pm 68.5 (1.3 - 675.4)	3	5	5	2	6	-	6	1	0	1	1	
<i>Medicago</i>	0.18	13	31 \pm 10.5 (0.2 - 100.0)	1	2	10	2	8	6	-	2	0	0	1	

types, and only 3 had 4 types of seed.

I found fecal pellets in 26 of 45 hibernation systems. Feces were found in 2 locations within the systems, either within the hibernaculum itself or in latrines located in the exit tunnel. Of 18 animals that died in hibernation and had fecal material in the hibernation system, only 1 had a latrine, whereas 3 of 8 animals that emerged had latrines. I found an average of 3 ± 0.5 fecal pellets with a combined mass of 0.16 ± 0.030 g in the hibernaculum chamber of 22 of 45 systems. In 17 cases, the fecal material was not obvious until the bedding had been sorted and cleaned, implying that it had not been collected in 1 place but had been incidentally distributed within the bedding. In an eighteenth instance, I found 1.74 g of feces (32 pellets) tucked beneath the bedding material within the chamber and clearly organized as a latrine. The remaining 4 hibernacula had much larger amounts of feces (2.3 ± 0.86 g, 43 ± 16.0 pellets) which were found strewn atop the bedding. Of 4 latrines in exit tunnels, 1 was a small pocket scratched 8 cm into the side of the exit tunnel and 3 were simply small (< 2 cm) depressions in the floor of the tunnel. Latrines were located 17 ± 9.1 cm from the lip of the chamber and contained 9.6 ± 2.63 g of fecal material (range: 4.7 - 15.8 g), with an average pellet count of 178 ± 48.6 (range: 88 - 293). The amount of feces found in any system was not significantly correlated with the period of time of post-immersion euthermy, the period of pre-emergence euthermy, the period of time post-emergence when the chamber was used as a sleep site, or the delay between the death or emergence of the animal and my excavation of the hibernaculum system. The mass of feces found did not differ between animals that had not completed hibernation and animals that had emerged, regardless of whether the feces were found in the hibernaculum

or in a latrine. Feces were found in 14 of 22 hibernation systems without a cache and in 12 of 23 systems with a cache, which was not significantly different from a random distribution ($\chi^2 = 0.61, P = 0.44$). The presence of feces was not correlated with any of the durations of euthermy after immergence or before emergence, total duration of euthermy, or time in hibernation ($r^2 \leq 0.12, F \leq 3.16, P \geq 0.09$).

DISCUSSION

Hibernation systems of Richardson's ground squirrels, until the animals emerge in the spring, are closed to the surface and not connected to other burrow systems, thus isolating the animals alone underground. The exit tunnel from the hibernaculum is excavated to 5.5 - 39 cm below the surface before torpor begins, but an exit hole to the surface is not excavated until the animal emerges in the spring. Hibernation systems of Richardson's ground squirrels always have a chamber (the hibernaculum) and an exit tunnel, usually have a drain tunnel, and often have 1 or more side tunnels. Based on the characteristics of each of these elements, I report evidence to support the prediction that Richardson's ground squirrels construct and, probably, gain access to the hibernation system via a connection from an active season burrow that is then usually closed off after the animal immerges.

I observed that 5 systems had unusual drain or side tunnels, and suggest that these were evidence of a connection between the hibernation system and an active-season system. In 4 of the 5 unusual tunnels I did not find either a chamber or a connection to the surface, so I cannot say conclusively that unusual tunnels were portions of other systems. Chambers and connections to the surface in conjunction with these unusual tunnels were

probably back-filled with soil removed from the hibernation systems, and I failed to discover them. I also failed to discover any connections to active season systems in the other 38 hibernation systems. Scheck and Fleherty (1980) described how *S. tridecemlineatus* thoroughly plugged burrows by firmly and repeatedly packing loads of soil into the old tunnels using their heads. Such packing would render the plugs difficult to find, although if the soil used for plugging was different than that of the surrounding soil, the plug should be apparent. Shaw (1925a) observed that relocation of soil by Columbian ground squirrels from one point in the hibernation system to another was easily detected due to differences in the soil strata through which the system had been excavated. Kawamichi (1989) commented that the soil used by Siberian chipmunks to plug the hibernation burrow was similar to that of the tunnel walls and concluded that it had been brought from nearby. The uniformity of soil at my study site likely contributed to my inability to find plugged burrow connections.

Mean V_h reported here for male and female Richardson's ground squirrels do not differ significantly from those reported by Michener (1992, Table 4) for animals on the same study site (males: $t_{43} = 1.50$, $P = 0.14$, females: $t_{20} = 0.70$, $P = 0.49$). The sexual difference, with hibernacula of males approximately twice the volume of those of females, is also consistent with her findings. Immersion mass of adult males did not differ between the 2 studies ($t_{12} = 0.69$, $P = 0.50$), but juvenile males in my study were significantly lighter in mass ($t_{48} = 3.45$, $P = 0.001$). Masses of females were not significantly different between the 2 studies (adult: $t_{46} = 0.76$, $P = 0.45$; juvenile: $t_{44} = 4.54$, $P = 0.13$). Mass-specific V_h did not differ for either males or females between this study and that reported by Michener

Table 4. Comparison of hibernaculum characteristics for 2 species of ground squirrels: *S. columbianus* at Pullman, Washington (Shaw, 1926a) and 2 elevations at Turner Valley, Alberta (Young, 1990), and 2 studies of *S. richardsonii* at Picture Butte, Alberta (Michener, 1992 and the present study). Original data were re-analyzed or weighted means were used to derive new means for comparison here. Data are thus presented as means, with standard error given only where original data were available. Age (J=juvenile, A= adult) and sex (M=male, F=female) classes presented vary according to the significantly different groupings in each species. Tmin = temperature minima at or near the depth of the hibernaculum. Additional data were taken from Young (1988) and Shaw (1925b). Data for *S. parryii* presented in the text were from different populations at different locations and so are not included here.

	<i>S. columbianus</i>									<i>S. richardsonii</i>			
	Shaw			Young						Michener		Present Study	
Elevation (m)	744			1470			2250			870		870	
Tmin (°C)	-3			-3.8 ± 0.6			-2.0 ± 0.8			-2.6		-3.4	
Age and sex	AM	AF	J	AM	AF	J	AM	AF	J	M	F	M	F
n	14	3	5	7	6	13	3	4	6	11	12	34	10
Depth to midpoint* (cm)	88 ± 3.9	61 ± 13.7	30 ± 10.7	93	75	60	54	67	43	56 ± 2.7	56 ± 2.7	57 ± 2.5	53 ± 4.7
Volume (L)	7.1 ± 0.5	2.8 ± 0.8	2.1 ± 0.4	9.8 ± 1.0	3.8 ± 0.4	2.9 ± 0.3	15.1 ± 0.6	5.7 ± 0.8	6.2 ± 1.7	10.5 ± 1.0	4.2 ± 0.4	8.7 ± 0.6	4.6 ± 0.4
Immergence body mass (g)**	756 ± 24	376 ± 7	329 ± 7	776 ± 24	570 ± 26	358 ± 10	865 ± 22	585 ± 22	375 ± 22	568 ± 8.8	426 ± 5.2	522 ± 11	455 ± 17
Mass specific volume (L/kg)	9.4	7.4	6.4	12.6	6.7	8.1	17.5	9.7	16.5	18 ± 1.8	11 ± 0.9	16.6 ± 1.1	10.2 ± 0.8

* Data from Shaw (1926a) and Young (1990) were adjusted to reflect measurement from centre of chamber to surface.

** Estimates of immergence body masses for squirrels studied by Shaw (1926a) were published in Young (1990).

(1992, males: $t_{43} = 0.64$, $P = 0.53$; females: $t_{20} = 0.65$, $P = 0.52$). Collectively, V_h for 45 males was 9.1 ± 0.5 L and for 22 females was 4.4 ± 0.3 L, and mass-specific V_h was 16.9 ± 0.9 L and 10.6 ± 0.6 L for males and females, respectively. The consistency in mass-specific volumes despite the differences in mass of the juvenile males between these 2 studies supports the use of mass-specific volumes to describe hibernacula in this species, rather than absolute volumes. Depths of hibernacula in the present study were identical to those reported by Michener (1992) in the same population.

A small variety of factors that may influence V_h and/or depth of the hibernaculum has been reported for *S. columbianus* living in montane meadows in Alberta and Washington state, and *S. parryii* living in sub-arctic and arctic Alaska. Hibernacula of *S. columbianus* were studied by Young (1990) at high and middle elevations at Turner Valley, Alberta, and by Shaw (1925a, 1926a) at low elevation at Pullman, Washington. For both Columbian and Richardson's ground squirrels, hibernacula of adult males were larger than those of adult females (Table 4). The magnitude of this difference is attenuated by calculating the mass-specific V_h , but the pattern of larger hibernacula of adult males is still present. Both Mayer (1953) and Barnes (1989) reported that V_h did not differ among sexes for *S. parryii* (based on reported diameters of 22 - 30 cm, the range for V_h for *S. parryii* is approximately 5 - 14 L). For *S. columbianus*, there is a trend that links increases in both V_h and mass-specific V_h with increasing elevation for all age and sex classes, but this is the only species for which data for populations at different elevations are available. V_h and mass-specific V_h in the present study both increase for male Richardson's ground squirrels with size of the seed cache, which confirms a report by

Michener (1993). Shaw (1926b) also reported that male but not female Columbian ground squirrels prepared caches, but his data on V_h were not linked to the size of the caches. *S. parryii* males are presumed to prepare caches whereas females are not (McLean and Towns 1981; Buck and Barnes 1999a), but there are no data published on cache size and no means of assessing any effect of cache size on V_h for arctic ground squirrels.

The animals in Young's (1990) high-elevation population of *S. columbianus* had shallower hibernacula than his lower elevation population, which he suggested was a result of a trade-off between reducing the costs of emerging from still-frozen soil in the spring and colder ambient temperatures during hibernation. Larger V_h allows an increase in the amount of insulation in the chamber, which reduces heat losses and offsets some of the costs incurred as a result of the shallower depth of the hibernaculum. The depth of the hibernaculum of *S. parryii* is restricted by the permafrost table (Buck and Barnes 1999b), and the animals therefore experience average soil temperatures of about -9°C during hibernation (Mayer 1953; Buck and Barnes 1999b). Although they cannot reach depths that will not freeze in winter, arctic ground squirrels apparently choose sites where the permafrost table is deeper than average (Buck and Barnes 1999b). These locations are in areas with shrubby vegetation that traps snow, and, as a result of the insulating snow cover, the soil is warmer than in areas without the vegetation. Buck and Barnes (1999b), based on calculations of the effect on heat loss of more nesting material in larger hibernacula, suggested that *S. parryii* may excavate the largest V_h that is practical for reducing heat loss regardless of the depth. V_h may therefore be less important than the depth of the hibernaculum in minimizing energy expense during hibernation in *S. parryii*.

On my study site, because there is little variation in the amount or persistence of the snow cover, or in the texture or moisture content of the soil across the site (pers. obs.), I suspect that temperature profiles of the soil are fairly uniform across the study site and any small variations in temperature of the soil are unlikely to be the source of much, if any, of the variability in V_h . Animals in deeper hibernacula would experience warmer temperatures than more shallow animals during the winter, although the converse would be true for the adult animals sequestering themselves in mid-summer, when deeper soils in early hibernation would be cooler than shallow soils. For Richardson's ground squirrels, there are no significant differences among age and sex classes for depth of the hibernaculum, and there is no correlation between depth of the hibernaculum and V_h . These facts suggest that Richardson's ground squirrels do not adjust V_h to reduce rates of heat loss.

Variations by age and sex in either V_h or hibernaculum depth differ depending upon the species examined and the location of the population. For Richardson's ground squirrels, both Michener (1992) and I found that V_h differed by sex but not by age, and hibernaculum depth did not differ at all by sex or age. Mayer (1953) and Barnes (1989) reported no differences among age and sex classes of arctic ground squirrels for either V_h or hibernaculum depth, but Buck and Barnes (1999b) did find that females hibernated in warmer burrows than males, and adults hibernated in warmer burrows than juveniles. Differences in V_h and hibernaculum depth between age and sex classes is more complicated for Columbian ground squirrels. Shaw (1926a) found that mass-specific V_h did not differ between age and sex classes, but there were some significant differences in hibernaculum depth between age and sex classes; adult males had significantly deeper

hibernacula than those of juveniles of either sex, but not than those of adult females, which were not different from those of the juveniles. V_h did not differ between age and sex classes for Young's (1990) high elevation population. However, in Young's (1990) low elevation population, V_h differed significantly between age and sex classes (adult males > adult females \geq juveniles). Significant differences in hibernaculum depth were also found between age and sex classes for Young's (1990) low elevation population (adult males > adult females > juveniles), but not for his high elevation squirrels, where frozen soils at emergence may influence squirrels to prepare shallower hibernacula. These differences across species and at different locations are difficult to interpret, but may relate to the ability of individual animals to compete for suitable locations at which to hibernate. Young (1990) suggested that juvenile Columbian ground squirrels compete with adults for hibernacula. They may often therefore be reduced to choosing hibernacula that are inferior in some way, which may explain the age-related differences he reported in hibernaculum depth and V_h at low elevation. Juvenile Richardson's ground squirrels immerse into hibernation 1 to 4 months later than adults and yet I found no difference between the hibernaculum systems of juveniles and those of adults. Suitable hibernaculum locations may not be limited on this site as is suggested by the observation that most Richardson's ground squirrels prepare a new hibernation system each season. The finding that there were age- and sex-related differences in the mean and minimum temperatures experienced by arctic ground squirrels (Buck and Barnes 1999b) may indicate that there is competition for suitable hibernation sites in that species. However, the amount of snow cover, and therefore the temperature of the soil, appears to be the important issue regarding competition for hibernation sites in *S.*

parryii, not the depth of the hibernaculum or V_h .

Why Richardson's ground squirrels excavate most of the exit tunnel before beginning torpor is unclear. Michener (1993) observed that male Richardson's ground squirrels resumed euthermia when the soil temperature at the depth of the hibernaculum was either already below 0°C and still declining or rising but still $<0^{\circ}\text{C}$. These squirrels might excavate the majority of their route to the surface before entering torpor and when soil is not frozen in order to avoid the much greater energy cost of excavation through frozen soil at the time of emergence. However, juvenile males in the present study emerged when soil temperature at the depth of the hibernaculum was already $>0^{\circ}\text{C}$, and female Richardson's ground squirrels resumed euthermia and emerged in March, when soil temperature at the depth of the hibernaculum is rising and already 0.5 to 3.0°C (Michener 1993; this report). Another explanation for pre-excavation of the exit tunnel is that opening a long tunnel, particularly one that rises nearer to the surface than the rest of the system, may improve the gas exchange of the closed hibernation system as a whole. If this is a factor, then one would predict that other squirrel species would also prepare most of the exit tunnel in advance of hibernation. Young (1990) did not find exit tunnels connected to the hibernacula of 4 Columbian ground squirrels (3 dead, 1 still hibernating) that he excavated at elevations of 1470 m and 2250 m. Shaw (1925a) did find that Columbian ground squirrels had pre-excavated exit tunnels at low elevation (744 m), which is similar to the elevation of my study site (870 m). Given that animals at higher elevations and therefore lower partial pressures of oxygen did not pre-excavate exit tunnels, it is questionable at this stage that gas-exchange is an important issue in determining whether the exit-tunnel is pre-

excavated. Information on when the exit tunnel is prepared by other species of *Spermophilus* may elucidate other factors that could dictate why the pre-excavation of an exit tunnel might be advantageous.

Drain tunnels have been found in hibernation systems of *S. columbianus* (Shaw 1926a; Young 1990), and in active-season burrows of *S. tridecemlineatus* (Johnson 1917; Wade 1930), *S. obsoletus*, and *S. franklinii* (Wade 1930). Shaw (1926a) also reported that those hibernation systems of *S. columbianus* without drains were constructed on hillsides and took advantage of the slope of the ground to protect the hibernaculum from water by virtue of an exit tunnel that dropped below the level of, and opened downslope from, the chamber. The provision of drainage for burrows, particularly on flat ground such as is found at the current site, would seem to have been strongly favoured in the selection of spermophiles in general.

The golden-mantled ground squirrel (*S. lateralis*) shows a preference for preparing active-season burrows that face southwest (Bihr and Smith 1998), perhaps to facilitate sun exposure and basking. This may also be true in the heliothermic yellow-bellied marmot (*Marmota flaviventris*), whose active-season burrows were found on southwest- or northeast-facing slopes (Svendsen 1976). Active-season burrows of arctic ground squirrels are oriented with entrances away from prevailing winds (Mayer 1955). The orientation of exit tunnels from hibernation systems to the north-northeast for the current population has no purpose for either drainage or sunning, given that there is no appreciable slope on the study site. Mayer (1955) explained that orientation of an entrance to a tunnel away from prevailing winds allows *S. parryii* to avoid the filling of their tunnels with wind-

borne debris, which would necessitate house-cleaning. This debris might also impede an animal's ability to detect above-ground movements of conspecifics and predators from within the tunnel. The orientation of the exit tunnel of Richardson's ground squirrels has no such purpose in a closed hibernation system. This orientation may reflect a general habit of the species to construct burrow away from the southern sun, or perhaps some other reason yet to be discovered.

Of 4 squirrels for which hibernaculum location was known in 2 consecutive years, none re-used the same hibernaculum for hibernation. Michener (1992, 2002) reported that of 21 pairs of years in which consecutive hibernaculum locations were known for 10 females and 4 males, only 2 females re-used the previous year's hibernaculum for hibernation. Juvenile male Richardson's ground squirrels spend 77% of the time in hibernation in torpor (Chapter III), a vulnerable physiological state during which the squirrel's ability to defend itself is limited, so selection of good quality hibernaculum sites should be favoured. Reuse of hibernacula might depend on the ease of excavation and availability of suitable sites for hibernation systems (Carl 1971). Young (1990) and Buck and Barnes (1999b) described a preference by *S. columbianus* and *S. parryii*, respectively, for areas where snow cover is greater (e.g. near bushes) in which to excavate their hibernacula, although Mayer (1955) contended that snow cover was not important for *S. parryii*. Buck and Barnes (1999b) showed that snow cover moderates the temperature regime of the hibernaculum, and they also found that *S. parryii* selects sites for hibernacula that have a deeper than average permafrost table. They also found that *S. parryii* does re-use hibernacula and this may reflect either the difficulty of excavating a new hibernation

system or that suitable locations are limited. The usual use of new hibernation systems each year by Richardson's ground squirrels suggests that the cost of excavating new hibernation systems is not prohibitively high and that suitable hibernaculum sites are not limited on this study site.

The use of underground hibernacula provides effective protection from some of the predators of Richardson's ground squirrels such as all raptors and coyotes, as well as from the harsh climatic conditions of southern Alberta winters. The habit of the vast majority of squirrels to avoid using the hibernaculum as a sleep site before hibernation reduces the labelling of the site with odours that other predators, such as weasels, snakes, and badgers, could use to locate the animals during hibernation. It also minimizes contamination of the nesting material with ectoparasites. The closure of the hibernation system eliminates the ability of non-burrowing predators such as weasels and snakes to gain access to the squirrels, and reduces the ability of burrowing predators such as badgers to find the animals during hibernation. However, it is evident in this study that neither closure of the system nor avoiding prior use of the hibernaculum as a sleep site are completely effective in protecting the animals from North American badgers. Closure of the system may also protect the occupant squirrel (and in the case of most males, the cache) from invasions by conspecifics.

The closure of the hibernation system also provides further protection from harsh climatic conditions by reducing air movement within the system. However, this also results in the dependence of Richardson's ground squirrels on diffusion of gases through the soil and therefore represents a potential limitation of closed systems. The inability to escape the hibernation system quickly in the event of sudden incidents, such as flooding, presents a rare

but real cost of closing the system to the surface. Presumably, the benefits of predator and ectoparasite avoidance and protection from the elements outweigh these potential costs.

The masses of grass bedding found in the hibernacula of Richardson's ground squirrels in this report were similar to those reported by Michener (1992) for males (250 ± 31 g vs. 327 ± 30 g, $t_{44} = 1.35$, $P = 0.18$) and slightly but significantly smaller for females (123 ± 18 g vs. 196 ± 28 g, $t_{19} = 2.16$, $P = 0.044$). Males, but not females, prepare hibernation caches in at least 4 species of ground squirrels: *S. richardsonii* (Michener 1992, 1993; this report), *S. columbianus* (Shaw 1926b), *S. saturatus* (Kenagy 1989), and *S. parryii* (McLean and Towns 1981; Buck and Barnes 1999a). This has been interpreted as the means by which males recover mass lost during hibernation (Michener 1992, 1993; Buck and Barnes 1999a), such that they are better able to meet the immediate demands of mate acquisition when females emerge. Barnes *et al.* (1986, 1987) and Michener (1992) also pointed out that testicular recrudescence and spermatogenesis occur rapidly only at normal body temperatures. The cache could meet the energy demands placed upon males that spend a period of time euthermic before emergence in the spring, during which testicular growth and spermatogenesis can be initiated.

Interpretation of the size of the seed caches is confounded by several factors. The average masses (292 g) and maximal masses (914 g) of caches found in this study are smaller than those found by Michener (1993; 552 g and 1079 g respectively) on the same site. Changes in agricultural crops adjacent to the study site from wheat and barley (both relatively large seeds) during the years of Michener's study to cicer milkvetch (a much smaller seed) during this study may explain this difference. Animals presumably need to

invest more time collecting a smaller seed; if time for collection of seed for caching is limited, then caches of smaller seeds would be smaller. Another factor is the question of how much of the originally cached seed remained when I excavated the hibernaculum. The quantity of seed I found may represent either an entire unused cache or the residual amount not yet consumed by the animal (Michener 1993). The absence of any seed in some hibernacula at the end of hibernation is unlikely due to the consumption of an entire cache. My finding of seed caches <5 g in 4 hibernacula suggests that it is unlikely that animals are able to consume all evidence of a seed cache. I am confident that no seed cache was prepared by any of the 10 females or by 12 of the 35 males. Likewise, Michener (1993) found no seed in the hibernacula of 12 females and those of 4 of 11 males.

I predicted that time in hibernation, time euthermic before the first torpor bout and/or time euthermic after the last torpor bout would be negatively correlated with the size of the seed cache found when I excavated the hibernaculum. The most parsimonious explanation for the lack of any such correlation is that the size of the seed cache originally prepared by the animals varies so greatly that it obscures any such relationships. Michener (1993) did indeed find that caches excavated in autumn ranged from 60 to 1736 g ($n = 8$), and I found 3 - 547 g in hibernacula of 9 males who had not completed hibernation. How much an animal has eaten by the end of the hibernation season is thus difficult to infer given the variation in initial masses of caches, but this issue is addressed in Chapter III. Despite this issue of not knowing how much of the original cache is unearthed at the end of hibernation, I found a positive correlation between the size of the remnant cache and the amount of total mass lost during hibernation. I suggest that animals that did not consume

more of the seed cache (and therefore had a larger remnant cache) recouped less mass than those that ate more of the cache. I interpret the positive correlation of remnant cache size with the rate of mass loss expressed per day of pre-emergence euthermy to suggest that pre-emergence euthermy is the time when the seed is consumed. This supports the inference made by Michener (1993) based on lower rates of mass loss per euthermy day for animals that had longer rather than shorter periods of pre-emergence euthermy.

Hibernation systems of Richardson's ground squirrels are single-purpose structures that are rarely used for anything but hibernation, and are rarely re-used. I have offered evidence to support the prediction that Richardson's ground squirrels access hibernation systems via connections to active-season systems, and confirmed that they excavate a drain tunnel to protect the hibernaculum and a blind-ending exit tunnel before entering torpor. Aside from confirming previous reports (Michener 1992, 1993) that males but not females cache seeds, and that males have larger hibernation chambers than females, no other sex-related differences were discovered, nor were any age-related differences detected. Based on the uniformity of most aspects of hibernation systems across age and sex classes despite differences in emergence schedules of the 4 age and sex classes and the tendency to use different sites for each subsequent hibernation season, Richardson's ground squirrels at my study site do not appear to face severe constraints on the availability of suitable hibernation sites.

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Chapter V

SUMMARY

I conducted a longitudinal study of Richardson's ground squirrels in hibernation over 2 seasons to determine the impact of seed-caching behaviour by males on body mass and body composition. The use of TOBEC to estimate body composition in Richardson's ground squirrels was evaluated in light of a number of factors purported to influence the accuracy of such estimates. Eartags, T_b , fullness of the gut, and hydration status had measurable effects on TOBEC readings, and thus on estimates of LBM and FBM. The increase in TOBEC reading due to the presence of eartags was easily assessed and subtracted from the original TOBEC reading to derive an adjusted TOBEC reading. Similarly, the slope of the relationship between T_b and the change in TOBEC was used to adjust TOBEC when $T_b \neq 37^\circ\text{C}$. An increase in mass by animals fed a laboratory diet after brief food deprivation was partitioned into both LBM and FBM. Because the effect involved both compartments, I suggested that effort should be invested to ensure that the issue of gut fullness during TOBEC measurements is addressed. An increase in mass in animals allowed to drink after water deprivation was partitioned entirely into LBM. Researchers interested solely in estimates of FBM can use TOBEC without being concerned about hydration status. However, the effect of hydration status on TOBEC must be taken into account if there is an interest in accurate estimates of LBM.

I derived an equation based on overwinter energy utilization and the proportional contributions of LBM and FBM to metabolism during hibernation to predict the amount of mass lost during hibernation. For animals that did not have seed caches (i.e., that relied

solely on endogenous energy stores for their metabolic needs during hibernation), there was no discrepancy between predicted and actual mass losses. Because the prediction was based on estimates of body composition using TOBEC and on estimates of VO_2 using metabolic data from Wang (1978, 1979), the accuracy of the prediction of mass loss validates the use of both techniques for Richardson's ground squirrels and may therefore be of value in other species if the same metabolic data are available.

Predicted mass loss significantly over-estimated actual mass loss for squirrels that did not have seed caches. Exogenous energy stores were not accounted for in the prediction equation, and the over-estimation was interpreted as evidence that animals had consumed some of the cache and thereby reduced the amount of mass lost during hibernation.

Male squirrels with and without caches in the hibernaculum lost mass at similar rates during hibernation. However, the rate of mass loss was significantly reduced per day of euthermy during hibernation for squirrels that had seed caches. The discrepancy between predicted and actual mass loss was correlated positively with the duration of the pre-emergence period of euthermy. This suggests that the timing of consumption of the cache occurs during that period, in the final days of hibernation. After hibernation, animals with caches had higher lean masses than males without caches. The facts that males with seed caches, when compared to males without seed caches, spent significantly longer in hibernation and yet had a significantly lower rate of total mass loss per euthermic day, and that the rates of LBM and FBM loss per euthermic day were nearly significantly lower suggests that males with caches recouped both LBM and FBM lost during hibernation.

These differences in total mass and LBM would theoretically confer the advantages of being larger and stronger during male-male competition for mate acquisition, and increased FBM would provide additional energy during mating. The cache may also provide enough energy to subsidize the costs of testicular recrudescence during pre-emergent euthermy.

Richardson's ground squirrels are sequestered during hibernation in systems that always have a nest chamber (the hibernaculum), a drain, and an exit tunnel, and may have 1 or more side tunnels. Three basic plans were identified to describe the arrangement of these tunnels and the chamber: the exit tunnel ran directly into the chamber with the drain tunnel arising as a level branch to the side of the exit tunnel; the exit tunnel ran directly into the drain with the chamber arising as a branch to the side of the exit tunnel; and a variation on the first plan in which the exit tunnel ran directly into the chamber with the drain arising as a branch to a dip in the exit tunnel.

Males had significantly larger hibernaculum volumes than those of females, with the difference explained by the larger mass of the males and the requirement for enough space to store a cache. Despite that difference, no other measurements of the hibernation systems were significantly different among the 4 age and sex groups. Richardson's ground squirrels always excavated the exit tunnel to a blind end before beginning torpor. Some larger systems are proposed as evidence that squirrels access the hibernation system via connections to active season systems that are closed before animals enter torpor.

Male Richardson's ground squirrels collected and stored a total of 8 different seed types. Barley, *Agropyron*, *Astragulus*, and *Medicago* formed the most frequent and most abundant seed types, with 1 - 4 species of seed found in each cache.

In summary, caching by male Richardson's ground squirrels appears to be a means of improving body condition such that caching squirrels recover both LBM and FBM lost during hibernation. Larger and fatter males may benefit from the investment of resources in preparing a cache at the end of one active season by improved mating success in the following year. Thus, seed caching by Richardson's ground squirrels is seen as a cost of reproduction.

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