

# Energy Expenditure and Testosterone in Free-Living Male Yellow-Pine Chipmunks

NED J. PLACE,<sup>1\*</sup> CLAUDIO VELOSO,<sup>2</sup> G.H. VISSER,<sup>3</sup> AND G.J. KENAGY<sup>1</sup>

<sup>1</sup>Department of Zoology and Burke Museum, University of Washington, Seattle, Washington 98195–1800

<sup>2</sup>Departamento de Ecología, Pontificia Universidad Católica de Chile, Casilla 114D, Santiago, Chile

<sup>3</sup>Center for Isotope Research (CIO), University of Groningen, 9747 AG Groningen, The Netherlands

**ABSTRACT** The onset of mating in yellow-pine chipmunks (*Tamias amoenus*) follows emergence from a prolonged period of energy conservation during hibernation. Energy expenditures are greatly accelerated to meet the demands of the reproductive season. When emerging from hibernation, typical male chipmunks (breeders) have enlarged testes and a high level of plasma testosterone (T). However, certain males that do not participate in reproduction (nonbreeders) maintain small testes and low plasma T levels and emerge several weeks later than the breeders. The timing of the terminal arousal from hibernation and onset of mating are associated with increased plasma T levels. Experimental elevation of T levels in *T. amoenus* outside the mating season has been associated with a decrease in body mass, further suggesting an effect of T on energy balance. To test this hypothesis, we measured daily energy expenditure (DEE) in free-living, nonbreeding male chipmunks in the presence and absence of a T-implant. We also measured DEE in breeding males when endogenous T levels were high. DEE of the nonbreeders was not affected by our manipulation of plasma T, and the DEE of breeding males did not differ from that of nonbreeders. We conclude that energy expenditure on a daily basis in male yellow-pine chipmunks is not influenced by levels of T. However, on a seasonal basis, the earlier emergence from hibernation by breeding males, which appears to be influenced by T, represents an overall seasonal energy expenditure that exceeds that of nonbreeding males. *J. Exp. Zool.* 292:460–467, 2002. © 2002 Wiley-Liss, Inc.

Behaviors associated with mating activities in male vertebrates (e.g., male-male aggression, courtship, and copulation with receptive females) result in increases in energy expenditure (Marler and Moore, '88; Michener and Locklear, '90). Expression of these behaviors is often dependent on activational effects of testosterone (T), which may generate the increases in energy expenditure. Effects of T on energy balance might be further emphasized in hibernating small mammals, because the timing of the terminal arousal from hibernation appears to be influenced by T (Lee et al., '90).

Reproductive male chipmunks and ground squirrels emerge from hibernation with high plasma T levels and enlarged testes (Barnes, '86; Holekamp and Talamantes, '91; Place and Kenagy, 2000). They also typically emerge a week or more before females and nonbreeding males (Michener, '84). In our study population of yellow-pine chipmunks, the incidence of nonbreeding males was low as all males usually become reproductively active (Place and Kenagy,

2000). However, in the present study, representing year 1999, 39% of males did not show testicular enlargement. Such years in which significant numbers of nonbreeding males are captured during the spring mating season are characterized by heavy winter snowfall and delayed snowmelt (Place, 2000a). The emergence of breeding males before females maximizes male mating opportunities in a polygynous system. The delayed emergence of nonbreeding males appears to be an energy-conserving strategy, employed to improve future reproductive success of younger individuals (Kenagy et al.,

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\*Correspondence to: Ned J. Place, Department of Psychology, University of California, Berkeley, 3210 Tolman, #1650, Berkeley, California 94720–1650. E-mail: ned@socrates.berkeley.edu

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'89). Additionally, thermal and temporal constraints of spermatogenesis require males to maintain euthermy for several weeks prior to emergence (Barnes et al., '86; Young, '90; Michener, '92), placing further energetic demands on males that prepare themselves to emerge early and begin mating.

Several hypotheses have been proposed that link reproduction and energy balance (Schneider and Wade, 2000), but few investigators have actually quantified energy expenditures associated with reproduction. In a study that measured energy expenditure in the field, Kenagy et al. ('89) found that total daily energy expenditure (DEE) of male golden-mantled ground squirrels (*Spermophilus saturatus*) was no higher during mating than it was after the mating season ended. That study did not directly test the effect of T on energy expenditure, but it does suggest a lack of effect on total daily expenditure, because plasma concentration of T was probably high during the mating period (Barnes, '86).

In the present study, we attempted a direct and experimental approach to describe the effects of T on energy expenditure by measuring DEE in nonbreeding male chipmunks in the presence and absence of exogenous T. We took advantage of a natural and unusual opportunity to manipulate T levels of a large cohort of males that showed delayed emergence and a lack of testicular enlargement. Several weeks prior to this experiment, we also measured DEE in naturally breeding males with enlarged testes.

We expected exogenous T to increase energy expenditure because of an earlier experiment with male chipmunks from this population that received T-implants in summer (after the breeding season); these experimentally T-implanted males showed a significant reduction in body mass (Place, 2000b). Similarly, Marler and Moore ('88, '89, '91) found body mass and survival decreased in T-implanted male lizards. Subsequently, Marler et al. ('95) specifically assessed the effect of T on daily energy expenditure in free-living mountain spiny lizards (*Sceloporus jarrovi*) and demonstrated a 31% increase in energy expenditure after T-implantation. In contrast, the ground squirrel study by Kenagy et al. ('89) suggests T does not have a marked effect on daily energy expenditure.

## MATERIALS AND METHODS

### *Animals and study site*

Individually marked male yellow-pine chipmunks were studied by live-trapping from March

1999 to June 1999 at a montane forest site (elevation 670m) in Chelan County, Washington. This population has been studied since 1995 (Kenagy and Place, 2000; Place and Kenagy, 2000). A survey of the study site on 15 March 1999, revealed 100% snow cover, and trapping was deferred until two weeks later. Weekly estimates of percent snow cover were continued until all snow had melted from the study site. Trapping began on 30 March 1999, on an area of 3.8 ha covered by a 14×14 grid of 196 Sherman live traps on 15-m centers. Owing to a decreased population density in spring 1999, the study site was expanded over an adjacent area of 1.3 ha covered by 100 traps on approximately 10-m centers to increase sample size. Traps were baited in the early morning with a bird seed mix and collected about 2 h later; study animals were brought to a central area to obtain blood samples for measurements of DEE and hormones. Chipmunks were weighed with a Pesola spring balance and examined for reproductive condition. Data on air temperature ( $T_a$ ) and snow depth at the National Climate Data Station (NCDS) in Plain, WA, located 5.2 km from our study site and at a similar elevation, were obtained from the NCDS website ([www.ncdc.noaa.gov/ol/climate/climatedata.html](http://www.ncdc.noaa.gov/ol/climate/climatedata.html)).

### *Daily energy expenditure*

Total daily energy expenditure (DEE, respiratory energy metabolism as  $\text{CO}_2$  production) was measured in the field by the doubly labeled water (DLW) method (Nagy, '87). A dose of approximately 6 ml/kg oxygen-18 (95 atom %) and hydrogen-2 (98 atom %), both in the form of water, was injected intraperitoneally, followed by an initial blood sample from the infraorbital sinus at least one hour later. Resampling of blood occurred on the following two days within  $\pm 2$ h multiples of 24h of the initial sample. We converted ml  $\text{CO}_2$  to kJ assuming  $21.7 \text{ J} \cdot (\text{ml } \text{CO}_2)^{-1}$ , based on respiratory quotient and plant diet (Cork and Kenagy, '89).

Eleven males in reproductive condition (fully enlarged, scrotal testes) were injected with DLW 19 to 21 April 1999; eight were recaptured one or two days later and resampled for DEE determinations. Seven nonbreeding males that emerged from hibernation several weeks later than the reproductively active males and showed no signs of testicular enlargement underwent DEE determinations at two later time points, in the presence and absence of T-implants. We were unable to measure DEE in T-implanted and control animals during the same time period because insufficient

numbers were caught. Additionally, the number of breeding males captured dwindled when our work with the nonbreeding males began. Thus, the study was limited to seven nonbreeding males that demonstrated the high rate of recapture necessary for DEE determinations. Nonbreeding males were implanted with silastic tubes filled with T during the mating season between 3 and 20 May (see next section). We injected DLW on 23 and 24 May, and we recaptured all seven animals one or two days later and resampled for DEE determinations. T-implants were removed after we obtained a set of blood samples for DEE analysis of T-implanted males. DLW injections were repeated in the same individuals (in the absence of T-implants) on 1, 2, and 9 June, and another set of blood samples was obtained one or two days later for DEE analysis.

#### *Implantation of testosterone*

The seven nonbreeding male chipmunks described above were captured, implanted, and released from 3 to 20 May 1999. After ethyl ether anesthesia, an implant was inserted subcutaneously through a small (5 mm) skin incision between the scapulae. The skin was sutured (0000 silk), and animals were returned to their trap site within 1 h after recovery. Implants were made of medical grade silastic tubing (10 mm total length; 1.98 ID/3.18 OD) and sealed with medical grade silicone sealant (Dow Corning, Midland, MI); implants were filled with 6mm of crystalline T (Sigma, St. Louis, MO).

#### *Hormone radioimmunoassays*

Plasma T and cortisol levels were measured on samples obtained from breeding males during the mating period and from nonbreeding males in the presence and absence of T-implants. Blood samples for hormone analysis were obtained from animals immediately after the final DEE sample had been collected. Blood was stored in a cooler for less than an hour and centrifuged; plasma was stored at  $-20^{\circ}\text{C}$  until radioimmunoassay. Plasma samples were thawed and assayed for testosterone as described by Wingfield and Farner ('75) and Ball and Wingfield ('87). Plasma samples of 70–100  $\mu\text{l}$  were extracted in dichloromethane, dried under nitrogen and reconstituted in 10% ethyl acetate: 90% isooctane. Steroids were separated via Celite (Sigma) chromatography, and recovery of T was measured for each sample. After separation, samples were dried under nitrogen, reconstituted in phosphate-buffered saline, and allowed to

equilibrate overnight. Samples were incubated with a primary antibody to testosterone (T-3003, Wien Laboratories, Succasunna, New Jersey) and tritiated hormone label (NEN Research Products, Boston, MA); dextran-coated charcoal was used to separate bound and free-labeled hormone. All samples were run in a single assay; intra-assay coefficient of variation was 3.7%.

Cortisol was measured using a solid-phase  $^{125}\text{I}$  radioimmunoassay (RIA) kit from DiaSorin (Stillwater, MN). Plasma samples were diluted fivefold with the serum blank provided and aliquotted in 10- $\mu\text{l}$  amounts into duplicate assay tubes. Cross-reaction of the cortisol antibody with corticosterone is  $< 0.4\%$ , as reported by the manufacturer. A dilution curve of chipmunk plasma was parallel to the standard curve of the kit. Cortisol was not detected in charcoal-stripped chipmunk plasma. Intra-assay coefficient of variation was 5.6%.

#### *Statistics*

Data were tested for normal distribution and homogeneity of variance. Results were analyzed with a commercial statistical program (Statview 5.0, SAS, Cary, NC) using paired and unpaired *t*-tests, Mann-Whitney 'U' test, and Wilcoxon Signed-Rank Test.

## RESULTS

When the first males emerged from hibernation in late March, snow cover remained extensive, with about 90% of the ground covered by snow at depths of 25cm–75 cm. Successful matings did not occur until mid-April, based on estimates of the earliest parturition in mid-May. The first capture of a female, on 15 April, is also consistent with these estimates. On the first day of trapping, 30 March, only three males were caught, and all were in reproductive condition, showing enlarged testes. Over the following two weeks, a cumulative total of 17 reproductive males emerged. Beginning on 19 April, nonbreeding males (unenlarged testes) began to emerge from hibernation, showing a median first capture of 7 May ( $n = 11$ ), compared to 14 April for reproductive males (Fig. 1). Thus, nonbreeding males comprised 39% of the male population.

The experiment was designed around a manipulation of T levels in the naturally occurring nonbreeding class of males. The T-implants raised the mean plasma T level of nonbreeding males more than an order of magnitude above the level measured following the removal of the implants (Table 1). The mean plasma T level of breeding

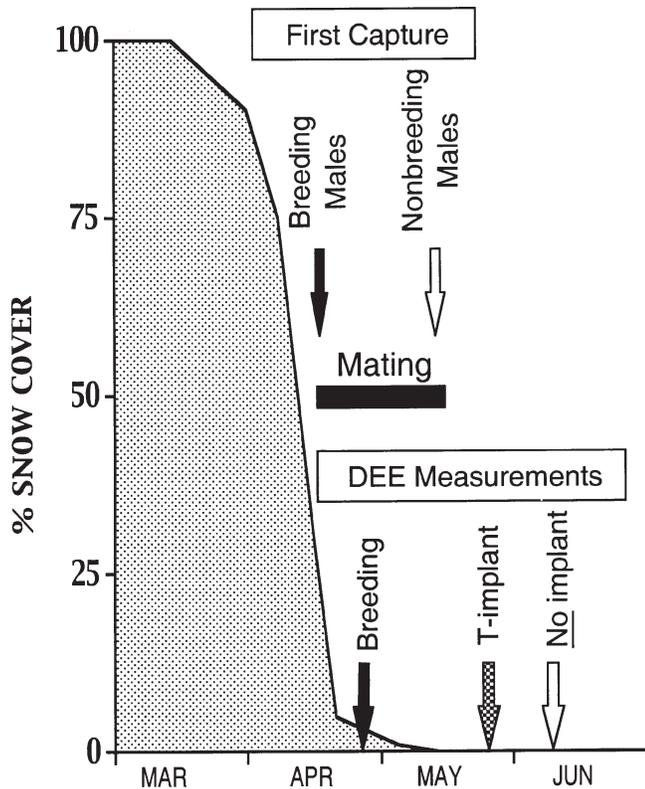


Fig. 1. Estimated percent snow cover at study site from March 1999 through June 1999. Duration of the mating season (bar) was based on dates of parturition and a gestation of 27d. Median first capture dates are displayed for breeding and nonbreeding males. Dates on which daily energy expenditure (DEE) measurements began are displayed for the groups of male chipmunks studied.

males was intermediate between that of untreated, nonbreeding males and T-implanted, nonbreeding males (Table 1). Mean plasma cortisol level of nonbreeding males was significantly depressed by about 10% when T-implants were present, compared to the cortisol level after implant removal (Table 1). Mean plasma cortisol level measured in breeding males during the mating season was significantly lower than the level measured in nonbreeding males caught several weeks later (*t*-test,  $P < 0.05$ ; Table 1). The difference in capture dates does not explain the lower plasma cortisol level of breeding males. When a subset of the breeding ( $n = 5$ ) and nonbreeding males ( $n = 4$ ) were captured during the final days of the mating season, 3 to 6 May, mean plasma cortisol level of breeding males remained significantly lower than that of nonbreeding males ( $992 \pm 134$  ng/ml and  $1500 \pm 104$  ng/ml, respectively; *t*-test,  $P < 0.05$ ).

Body mass of nonbreeding males did not change

TABLE 1. Plasma levels (ng/ml) of testosterone (T) and cortisol of nonbreeding male chipmunks ( $n = 7$ ) with and without T-implants

Reproductive status	T-implant	Testosterone	Cortisol
Nonbreeding	Present	$4.9 \pm 0.5$	$1253 \pm 65$
	Absent	$0.2 \pm 0.02$	$1406 \pm 91$
		$P < 0.01$	$P < 0.01$
Breeding	—	$2.0 \pm 0.3^a$	$795 \pm 60^b$

Note: Values are means  $\pm$  SD. Superscript *a* denotes T levels of breeding males are intermediate to T levels of nonbreeding males, significantly lower when T-implants are present and significantly higher when T-implants are absent ( $P < 0.05$ ). Superscript *b* denotes cortisol levels of breeding males are significantly lower ( $P < 0.05$ ) than cortisol levels of nonbreeding males, regardless of T-implant presence or absence. Tests: Wilcoxon signed and rank paired *t*-tests were used to compare T and cortisol levels of nonbreeding males, respectively. Mann-Whitney 'U' and *t*-tests were used to compare T and cortisol levels, respectively, of breeding and nonbreeding males. Values for breeding males ( $n = 8$ ) provided for comparison.

significantly after T-implant insertion, but after implant removal body mass increased about 3% ( $P = 0.05$ ; Table 2). Despite the change in body mass associated with implant removal, DEE of nonbreeding males was not affected by the presence or absence of T-implants ( $P = 0.30$ ; Table 2). On the other hand, DEE of breeding males was greater than that of T-implanted, nonbreeding males (Mann-Whitney 'U' test,  $P = 0.028$ ), but after implants were removed, DEE did not differ ( $P = 0.30$ ). Pairwise comparisons of mean mass-specific DEE were statistically identical to those for mean whole-animal DEE.

Environmental conditions were similar during the two periods when DEE measurements were obtained in nonbreeding males. No precipitation fell, and the range of overnight minimum air temperatures ( $T_{a-min}$ ) were similar ( $-0.6^\circ\text{C}$  to

TABLE 2. Daily energy expenditure (DEE) and body mass of nonbreeding male chipmunks ( $n = 7$ ) with and without T-implants

Reproductive status	T-implant	DEE (kJ/d)	Body mass (g)
Nonbreeding	Present	$80.9 \pm 5.0^a$	$42.0 \pm 0.5$
	Absent	$85.0 \pm 13.8$	$43.3 \pm 0.9$
		$P = 0.30$	$P = 0.05$
Breeding	—	$92.5 \pm 11.5$	$42.7 \pm 0.5$

Note: Values are means  $\pm$  SD. Superscript *a* denotes mean DEE of nonbreeding males with T-implants present is significantly different ( $P < 0.05$ ) from DEE value of breeding males; body mass of nonbreeding males does not differ from that of breeding males, regardless of T-implant presence or absence. Tests: Wilcoxon signed rank and paired *t*-tests were used to compare DEE and body mass of nonbreeding males, respectively. Mann-Whitney 'U' and *t*-tests were used to compare DEE and body mass, respectively, of breeding and nonbreeding males. Values for breeding males ( $n = 8$ ) provided for comparison.

11.7°C with T-implants vs. -1.1°C to 11.7°C without). Maximum daytime air temperatures ( $T_{a-max}$ ) were generally higher and less variable when DEE was measured with T-implants (24.4°C to 29.4°C) than without (13.9 to 22.2°C). When DEE measurements were obtained in breeding males, overnight  $T_{a-min}$  ranged from -2.2°C to 0.6°C, and daytime  $T_{a-max}$  ranged from 12.2°C to 22.8°C. Air temperatures were most disparate between times of comparison between breeding males and T-implantation of nonbreeding males; this may have contributed to the difference in DEE values between these groups.

### DISCUSSION

The general observation that male chipmunks in reproductive condition had DEE values comparable to those of nonbreeding males suggests that T does not affect the total energy expenditure in a 24-hr period. Likewise, male golden-mantled ground squirrels had similar DEE during and after the breeding season (Kenagy et al., '89). Additionally, our experimental elevation of T in male chipmunks had no effect on DEE of nonbreeding individuals.

In both yellow-pine chipmunks and spiny lizards, exogenous T has been associated with a decrease in body mass, suggesting a negative energy balance (Marler and Moore, '88; Place, 2000b). The effect of exogenous T on energy balance consisted of an increase in DEE of spiny lizards (Marler et al., '95), but apparently not in the chipmunks (present study). Physiological differences related to the phylogeny of mammals and reptiles are likely to explain some of the different effects of T on DEE in chipmunks and lizards. The nighttime metabolism of a diurnal, ectothermic lizard is much lower than its daytime metabolism. By comparison, endotherms such as chipmunks maintain a high metabolic rate and body temperature at night. Because the daytime energy expenditure of an ectotherm is so large compared to the nighttime energy expenditure, it follows that total DEE of a lizard is more sensitive to changes in daytime activity behavior than is the total DEE of a mammal. Additionally, *S. jarrovi* aggressively defend territories during breeding (Marler and Moore, '88), which is consistent with increased DEE; whereas *T. amoenus* occupy overlapping home ranges and are not known to be territorial, even during the mating season (Broadbooks, '58). Thus, one would not expect an increase in total daily energy expenditure in chipmunks associated with territoriality.

Differences in the design of the present study and the Marler et al. ('95) study (time of year and reproductive state of animals when T-implants were given) may also contribute to the disparate results. Nonbreeding male chipmunks were given T-implants at the end of the mating season (present study), but the mountain spiny lizards were in reproductive condition when they received T-implants during the mating season. Ketterson et al. ('91) noted that the effect of T-implantation on body mass of dark-eyed juncos (*Junco hyemalis*) varied with the timing of insertion. Body mass decreased significantly in birds implanted before mating, while no change in body mass occurred when birds were implanted during mating. Similarly, when yellow-pine chipmunks were implanted with T during late summer, body mass decreased significantly one month after implantation (Place, 2000b). But, in the present study, T-implantation during late spring did not affect body mass. However, potential changes in body mass were assessed after a shorter interval in the present study (median 19d) than they were in the previous study (median 28d; Place, 2000b). The interval between implant insertion and removal in the present study may have been too short for T to affect body mass significantly. Body mass increased after implant removal, but, in fact, it increases naturally at this season (late spring) in *T. amoenus* males (Place and Kenagy, 2000). Thus, social context, duration of implantation, season, and environmental conditions in which T was implanted make it difficult to compare the effects of T on energy balance across these species and studies.

The fact that 39% of male chipmunks were nonbreeders in the year of this study reflects the idea that preparation for and involvement in mating are energetically demanding. These demands are more easily met under favorable environmental conditions, as noted in other years when essentially all males captured demonstrated reproductive readiness (Kenagy and Barnes, '88; Place and Kenagy, 2000). Nonbreeding males probably gain their greatest energy savings by delaying their emergence from hibernation. Emergence of nonbreeding males later than breeding males was noted in chipmunks in the present study and in ground squirrels in previous studies (reviewed in Michener, '84). We estimated the energy expenditure of nonbreeding male chipmunks during the additional 22d they hibernated as 325 kJ and compared this value to the energy expenditure of males that emerged earlier, in reproductive condition (2035 kJ, see appendix). Nonbreeding males

were assumed to have aroused from torpor on five occasions (Geiser et al., '90) and to have remained euthermic for 12 h at each arousal. Thus we assumed that torpor continued for the remaining 19.5d and yielded a substantial energy savings despite the energetic demands of arousal. The energy expenditure of nonbreeding males in hibernation represents only 16% of the energy used by continuously euthermic reproductive males in the same period. This closely approximates the proposed overwinter energy savings attributed to hibernation in ground squirrels, estimated as 20% or less of the total of an animal that remains euthermic (Wang and Hudson, '71; Kenagy, '86). Overall, the energy savings from delayed emergence by nonbreeding males may translate into a survival advantage and an increased potential for future reproductive success.

The major energy savings associated with a lack of reproductive effort occur prior to emergence from hibernation. Low body temperature associated with torpor inhibits testicular growth and spermatogenesis (Barnes et al., '88). To emerge from hibernation burrows ready to mate at the earliest opportunity, males must make their terminal arousal about a month before emergence. In field studies, Young ('90) and Michener ('92) showed that reproductive male ground squirrels establish a euthermic state one to three weeks before females and nonbreeding males. Moreover, experimental elevation of T in the laboratory accelerates the timing of the terminal arousal in golden-mantled ground squirrels, *Spermophilus lateralis* (Lee et al., '90). In yellow-pine chipmunks, complete enlargement of the testes of animals held in the laboratory at 5°C was achieved, on average, 23d (range 12d to 31d) after the terminal arousal (Place, unpublished). Reduced overwinter survival of T-implanted chipmunks in the field indirectly suggests that T disrupts hibernation in chipmunks (Place, 2000b), as it does in ground squirrels in the laboratory (Lee et al., '90). In the present study, most of the nonbreeding male chipmunks were probably yearlings, but at least one individual was known to be an older adult that had enlarged testes in the preceding spring. Additionally, in previous years when emergence followed winters milder than that of the present study, nearly all males, including yearlings, demonstrated reproductive readiness (Place and Kenagy, 2000). The tactic of reproductive males, regardless of age, is to emerge before females.

The timing of the commitment by a male yellow-pine chipmunk to become reproductively ac-

tive or not is uncertain. This commitment could be made as animals enter hibernation (immersion) or sometime during the months of hibernation. Barnes ('84) suggested that the level of energy stores at the time of immersion is an important factor in golden-mantled ground squirrels, as pre-immersion food restriction reduced body mass and prevented reproductive activation in *S. saturatus*. Chipmunks and ground squirrels differ in important ways with regard to their overwinter strategies; for example, the active season of chipmunks may be two to three months longer. In addition, chipmunks rely on cached food for fuel throughout the winter, while ground squirrels generally rely on body fat stores (Kenagy and Barnes, '88). The longer active season means that juvenile chipmunks have more time to match the body mass of adult males prior to immersion (Kenagy and Barnes, '88; Place and Kenagy, 2000); whereas juvenile ground squirrels sometimes weigh significantly less than adult males in late summer (Kenagy and Barnes, '88). Thus, cache size at immersion, and not body mass, may be a better predictor of an individual chipmunk's potential for achieving reproductive readiness. How and when chipmunks assess the adequacy of their external energy stores (cache) for a potential reproductive effort that may be months ahead are intriguing and challenging questions. By maintaining low T levels and avoiding an early terminal arousal from torpor and an early emergence from hibernation, nonbreeding chipmunks conserve energy. The daily activities actually associated with mating, and by inference T, do not appear to be unduly costly in male yellow-pine chipmunks, but the details of testosterone's effects on energy expenditure remain to be explored more fully.

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## APPENDIX

Energy expended during 22d of hibernation by nonbreeding male chipmunks (328 kJ) was estimated by summing energy used during 19.5d of torpor (17 kJ) and five arousals totaling 2.5d (311 kJ). Time required to arouse from torpor and raise body temperature from 5°C to 38°C was based on a rate of 0.8°C · min<sup>-1</sup> (Cade, '63) and totaled 2.5h. Euthermic activity at 5°C following each arousal accounted for the remainder of the time when animals were not in torpor (57.5h). Calculations were based on the following assumptions:

1. Energy expenditure expressed in kJ after converting from oxygen consumption and assuming 20.1 kJ · (l O<sub>2</sub>)<sup>-1</sup>.
2. Energy expenditure during 19.5d of torpor (16.6 kJ) was calculated using MR of 0.042 mlO<sub>2</sub> · g<sup>-1</sup> · h<sup>-1</sup> (Geiser, '88).
3. Torpor bout length was 5d, yielding five arousals in 22d (Geiser et al., '90).
4. Energy expended for each arousal (18.5 kJ) was calculated by multiplying basal metabolic rate (BMR) of 1.45ml O<sub>2</sub> · g<sup>-1</sup> · h<sup>-1</sup> (Kenagy and Vleck, '82) by the equivalent increase in MR based on a Q<sub>10</sub>≈3 (Wang and Hudson, '71; Malan, '78; Geiser, '88; Geiser et al., '90).
5. Burrow temperature of 5°C in March/April was based on soil temperature measurements from Kenagy et al. ('89). Calculations for MR and energy expenditure (3.8 kJ · h<sup>-1</sup>) during the period of euthermia within the burrow following each arousal was based on the equation MR = -0.134 · (T<sub>a</sub> = 5°C) + 5.186 (Heller and Gates, '71).