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Original Investigation

Age and sex differences in hibernation patterns in free-living Anatolian ground squirrels

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ABSTRACT

Anatolian ground squirrels, *Spermophilus xanthoprymnus* (Bennett, 1835), are nearly endemic to Turkey. Various aspects of the biology of Anatolian ground squirrels have been studied. However, a detailed description of hibernation, with special attention to age and sex differences, has not previously been reported. Thus, in this study, we aimed to present a detailed description of hibernation in a population of free-living Anatolian ground squirrels, with special attention to age and sex differences, in relation to soil temperature (Tsoil) at the expected depth of the hibernacula. This study is the first such study for Old World ground squirrels (the genus *Spermophilus* sensu stricto) and incorporates an additional species from a different environment (e.g. warmer than those of others) into comparative studies of hibernation patterns in Holarctic ground squirrels (the tribe Marmotini) under natural conditions. Body temperature (Tb) was continuously recorded from late summer to spring by intraperitoneally implanted temperature loggers and Tsoil by a temperature logger at a depth of 1 m in the field. Anatolian ground squirrels spent about half of the year in hibernation. However, differences in timing of the beginning and end of hibernation resulted in differences in the duration of hibernation among age-sex classes. The duration of torpor bouts was short at the beginning of hibernation, increased as Tsoil decreased, reached its maximum toward the end of hibernation, and decreased thereafter. Adult males exhibited hibernation characteristics rather different from those of other age-sex classes, spending less time in hibernation, exhibiting shorter torpor bouts and longer interbout arousals, especially toward the end of hibernation, and spending proportionally less time in torpor. In conclusion, we observed that hibernation patterns in free-living Anatolian ground squirrels were influenced by age-sex classes, as well as environmental conditions.

Introduction

From an ecophysiological point of view, animals must maintain a long-term balance between energy income and energy expenditure (McNab, 1974). This balance is particularly challenging for small mammals that require large amounts of energy to maintain their high metabolism (Aschoff, 1981). Moreover, especially in small mammals of the temperate zone, the energetic cost of maintaining high metabolism is exacerbated during winter months when both ambient temperature (Ta) and the quantity and/or quality of food resources are low (Bartholomew, 1982; Speakman, 2000). Many small mammals overcome these energetic constraints by employing torpor, a temporary and controlled reduction of metabolism, body temperature (Tb), and other physiological functions, as an energy-saving strategy (Lyman, 1982; Heldmaier and Ruf, 1992). Expression of torpor can be highly flexible among species and even among individuals of a single species, although two common patterns of torpor have been most commonly recognized: daily torpor (lasting < 24 hours) and hibernation (Geiser and Ruf, 1995; Ruf and Geiser, 2014). Hibernation consists of a sequence of multiday torpor bouts, during which metabolism decreases significantly below basal metabolic rate and Tb is often lowered, universally interrupted by brief interbout arousals (Pengelley and Fisher, 1961; Pohl, 1961; Wang, 1979; Geiser, 1988; Ruf and Geiser, 2014).

Anatolian ground squirrels, *Spermophilus xanthoprymnus* (Bennett, 1835), are group-living, diurnal, hibernating, and predominantly herbivorous, burrowing ground-dwelling squirrels (Kryštufek and Vohralík, 2005; Kart Gür and Gür, 2010). They are nearly endemic to Turkey, present in central and eastern (especially northeastern) Anatolia, with minor range extensions into Armenia and northwestern Iran. Anatolian ground squirrels associate with a continental climate, i.e. a drier climate with greater seasonality, and inhabit short-grass steppes (Kryštufek and Vohralík, 2005,

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2012; Kart Gür and Gür, 2010; Gür, 2013). In central Anatolia, they hibernate individually in underground burrows, from about late summer or early autumn (August or September) to late winter or early spring (February or March). Females mate shortly after emergence from the hibernacula and wean only one litter each year. Parturition occurs in April and juveniles appear aboveground in May. Anatolian ground squirrels appear to rely mainly on fat reserves stored during the active season as the source of energy overwinter and therefore dramatically lose body mass during hibernation (Gür and Kart Gür, 2005; Kart Gür and Gür, 2010).

Various aspects of the biology of Anatolian ground squirrels have been studied (Yiğit et al., 2000; Kart Gür and Gür, 2010 and references therein). However, although daily rhythmicity of Tb before and during hibernation under both natural and laboratory conditions was examined using intraperitoneally implanted temperature loggers (Kart Gür et al., 2009), a detailed description of hibernation, with special attention to age and sex differences, has not previously been reported. Thus, in this study, we aimed to present a detailed description of hibernation in a population of free-living Anatolian ground squirrels, with special attention to age and sex differences, in relation to soil temperature (Tsoil) at the expected depth of the hibernacula. This study is the first such study for Old World ground squirrels (the genus *Spermophilus* sensu stricto; Helgen et al., 2009), because previous studies were performed under either semi-natural (*Spermophilus citellus*; Hut et al., 2002 – a sister species of Anatolian ground squirrels) or laboratory conditions (*S. dauricus*; Yang et al., 2011). Moreover, this study incorporates an additional species from a different environment (e.g. warmer than those of others; see Fig. 5) into comparative studies of hibernation patterns in Holarctic ground squirrels (the tribe Marmotini; Thorington et al., 2012) under natural conditions and therefore extends the understanding of how hibernating mammals respond to the climatic and ecological conditions of the environment in which they live. Owing to previous findings on differences in autumnal and spring behaviors and requirements for reproductive maturation of males and females in hibernating species of ground squirrels (Williams et al., 2014 and references therein; Gür and Kart Gür, 2005; Kart Gür and Gür, 2010), we hypothesized that hibernation characteristics would vary substantially among age-sex classes.

Material and methods

Ethics statements

This study was carried out according to the "Guidelines for the Capture, Handling, and Care of Mammals as Approved by the American Society of Mammalogists" (Animal Care and Use Committee, 1998) and the "Guide for the Care and Use of Laboratory Animals" (Institute for Laboratory Animal Research-ILAR, 1996), and approved by the Local Ethical Committee for Animal Care and Use of Hacettepe University School of Medicine, Ankara, Turkey.

Study area

The study area is a short-grass steppe landscape located in the dry forest-anthropogene steppe subregion of the central Anatolian continental ecoregion (Atalay and Mortan, 2006), about 50 km south of Ankara, Turkey (39.48 N, 32.85 E, 1 190–1 205 m altitude). In the study area, mean annual air temperature is 10.0 °C, with the warmest quarter from June to August (mean = 20.2 °C) and the coldest quarter from December to February (mean = −0.4 °C). Mean annual precipitation is 399 mm, with the wettest quarter from March to May (mean = 134 mm) and the driest quarter from July to September (mean = 44 mm).

Field studies

The field studies were conducted from April 2005 to May 2007 in a population of free-living Anatolian ground squirrels. Individuals were trapped using treadle-style, wire-mesh live traps (Collapsible Traps with One Trap Door, Code: 202; Tomahawk Live Trap Co., Tomahawk, WI, USA) baited with peanut butter, placed at burrow entrances. At first capture, Anatolian ground squirrels were marked permanently with a numbered metal tag in each ear (National Band and Tag Co., Newport, KY, USA) and their fur was marked with a commercial hair dye in individually recognizable patterns. At each capture, date, location, tag number, age, sex, body mass (±5 g, Pesola spring scale; Pesola AG, Rebmattli, Baar, Switzerland), and reproductive status were recorded. Individuals were identified as juvenile if trapped during the active season of their birth year and as adult after their first hibernation. Males were defined as reproductive if they had testes descended into a pigmented scrotum. Females were defined as pregnant if they had enlarged, darkly pigmented teats and as lactating if they had large, lightly pigmented teats with surrounding fur flattened (Gür and Kart Gür, 2005; Kart Gür and Gür, 2010).

Towards the end of the active season (on multiple dates from 13 June to 22 August of 2005 and 2006), 37 free-living Anatolian ground squirrels chosen for implanting temperature loggers were trapped and then released to the field after implantation of the loggers. At the beginning of the active season (on multiple dates from 21 February to 26 April of 2006 and 2007), 23 (62%) of 37 free-living Anatolian ground squirrels (three adult males, nine adult females, four juvenile males, and seven juvenile females) implanted with temperature loggers were re-trapped and then released to the field after removal of the loggers. Because of the risk of predation in the field (Gür and Barlas, 2006), these individuals were trapped within a few days after emergence from the hibernacula.

Tsoil was recorded by a temperature logger (DS1922L; for specifications, see below) at a depth of 1 m in the field. This depth was chosen, because it is the expected depth of the hibernacula of Anatolian ground squirrels (Karabağ, 1953; Kart Gür et al., 2009).

Temperature loggers

Tb of free-living Anatolian ground squirrels was recorded by two types of temperature loggers: Thermochron iButtons (DS1922L, ∼3 g, range −40 to 85 °C, resolution 0.0625 or 0.5 °C; Maxim Integrated Products, Inc., Sunnyvale, CA, USA) or StowAway Tidbits (customized temperature data logger, 8.7 g, range −4 to 44 °C, resolution 0.16 °C; Onset Computer Corporation, Bourne, MA, USA). Adults were surgically implanted with Thermochron iButtons and juveniles with StowAway Tidbits. The loggers were programmed to record Tb at 15-min (StowAway Tidbits) or 50-min (with a resolution of 0.5 °C; Thermochron iButtons) intervals. All other details of preparation of the loggers were described by Kart Gür et al. (2009).

Implantation and removal of temperature loggers

Free-living Anatolian ground squirrels were transported from the field to the laboratory at Hacettepe University, Ankara, Turkey, at a distance of approximately 60 km. Temperature loggers were surgically implanted into or removed from the peritoneal cavity of individuals under sterile conditions and general anesthesia. After recovery, individuals were released to the field at the site where they were trapped. All other details of implantation and removal of the loggers were described by Kart Gür et al. (2009).

Table 1
Summary of hibernation patterns in Anatolian ground squirrels by age-sex class. Different letters within a parameter indicated statistical differences among age-sex classes. Descriptive statistics were presented as mean ± SE and range.

Parameters	Adult males	Adult females	Juvenile males	Juvenile females	*F*-statistics
n	3	9	4	7	
Beginning of hibernation (date)	31 Aug ± 6.7 a 22 Aug–13 Sep	15 Aug ± 4.9 b 04 Aug–18 Sep	10 Sep ± 2.9 a 03–17 Sep	09 Sep ± 1.8 a 02–16 Sep	$F_{3,19} = 8.8^{***}$
End of hibernation (date)	14 Feb ± 3.5 a 08–20 Feb	10 Mar ± 2.2 b 02–22 Mar	09 Mar ± 4.8 b 02–23 Mar	27 Mar ± 3.5 c 16 Mar–09 Apr	$F_{3,19} = 18.7^{***}$
Spring body mass (g)	252 ± 12 a 235–275	182 ± 6 b 145–205	175 ± 12 b 160–210	156 ± 7 b 130–190	$F_{3,19} = 16.5^{***}$
Total duration of hibernation (days)	167.5 ± 3.7 a 160.2–171.7	207.2 ± 5.3 b 168.6–222.6	180.2 ± 6.3 a 166.2–195.6	198.9 ± 3.0 b 185.0–209.1	$F_{3,19} = 9.8^{***}$
Mean duration of torpor bouts (days)[a]	6.3 ± 0.3 a 5.8–6.6	7.7 ± 0.2 b 6.7–8.3	8.6 ± 0.3 b 8.0–9.5	8.0 ± 0.3 b 7.2–9.7	$F_{3,19} = 7.2^{**}$
Mean duration of interbout arousals (days)[a]	1.14 ± 0.05 a 1.08–1.24	0.92 ± 0.02 b 0.86–0.99	0.91 ± 0.06 b,c 0.83–1.09	0.77 ± 0.05 c 0.67–1.05	$F_{3,19} = 9.8^{***}$
Duration of the longest torpor bout (days)	14.0 ± 1.4 a 11.9–16.6	17.6 ± 0.6 b 14.9–20.2	18.8 ± 0.3 b 18.0–19.6	18.1 ± 0.7 b 16.3–22.0	$F_{3,19} = 4.7^{*}$
Number of torpor bouts	22.7 ± 1.2 a 21–25	24.1 ± 0.7 a 20–28	19.0 ± 0.4 b 18–20	22.7 ± 0.8 a 20–26	$F_{3,19} = 6.1^{**}$
Total duration of torpor bouts (days)	142.9 ± 2.3 a 138.3–145.9	186.1 ± 5.0 b 149.8–199.1	164.0 ± 7.0 c 147.7–180.5	182.0 ± 3.4 b 168.9–194.8	$F_{3,19} = 11.1^{***}$
Total duration of interbout arousals (days)	24.6 ± 1.4 a 21.8–26.1	21.1 ± 0.6 b 18.8–23.4	16.2 ± 0.8 c 15.1–18.5	16.9 ± 1.0 c 12.7–21.1	$F_{3,19} = 14.5^{***}$
Total duration of torpor bouts as % of hibernation	85.3 ± 0.5 a 84.7–86.4	89.8 ± 0.3 b 88.9–91.0	90.9 ± 0.7 b,c 88.9–92.3	91.5 ± 0.5 c 89.2–93.9	$F_{3,19} = 22.2^{***}$
Lowest minimum steady-state Tb (°C)	7.1 ± 0.5 a 6.6–8.1	5.4 ± 0.4 a,b 3.6–7.6	4.6 ± 0.4 b 3.7–5.5	3.7 ± 0.6 b 1.7–6.2	$F_{3,19} = 5.9^{**}$

[a] Based on mean value throughout hibernation for each individual.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Data and analyses

Tb records of 23 free-living Anatolian ground squirrels were analyzed for the parameters given in Table 1 (for further details, see Fig. 1 and "Results" section; note that the data used here were also used in Kart Gür et al., 2009 that focused on daily rhythmicity of Tb). These parameters were obtained using an Excel-based macro written in-house. Hibernation was defined as the period from initiation of the first multiday torpor bout to termination of the last multiday torpor bout during which individuals remained continuously underground in the hibernacula (Kart Gür et al., 2009; Ruf and Geiser, 2014; Fig. 1). Thus, in this study, we focused on hibernation, but not on below-ground euthermic intervals immediately before and after hibernation, because we lack observational data, especially on immergence into the hibernacula. However, sometimes the period from initiation of the first multiday torpor bout to termination of the last multiday torpor bout is referred to as heterothermy, and the period during which individuals remained continuously underground in the hibernacula is referred to as hibernation (e.g. Michener, 1992). A torpor bout was defined as the period from the first point when Tb was <30 °C to the first point when Tb was >30 °C and therefore an interbout arousal as the period from the first point when Tb was >30 °C to the first point when Tb was <30 °C (Fig. 1; Young, 1990; Michener, 1992; Ruf and Arnold, 2000; Zervanos and Salsbury, 2003; Buck et al., 2008; Lee et al., 2009; Healy et al., 2012). If Tb remained <30 °C for >24 hours, it was defined as a multiday torpor bout and if Tb remained <30 °C for <24 hours, it was defined as a short torpor bout (Ruf and Geiser, 2014).

Anatolian ground squirrels were grouped by age and sex, i.e. adult and juvenile males and females. Each individual was represented once in the data analysis, except in the case that all observations from all individuals were combined to examine the relationship between the duration of torpor bouts and minimum Tb. Using a two-way (year × age-sex class) univariate ANOVA model (Sokal and Rohlf, 1995), we found no effect of year or interaction between year and age-sex class on any parameter of interest. Thus, data from both years, i.e. 2005–2006 and 2006–2007 hibernation seasons, were combined to increase sample size. It is important to note here that population density, especially of adult males, and inter-year survival, especially of males, are relatively low in this population (Gür and Barlas, 2006; Kart Gür and Gür, 2010), partly justifying combining data from both years and small sample sizes, especially for males. Variation among age-sex classes in any parameter of interest was examined by means of a one-way (age-sex class) univariate ANOVA model and multiple comparisons for each parameter were performed using a post hoc Duncan's multiple-range test (Sokal and Rohlf, 1995). Other statistical tests (Fisher's exact test, one-sample *t* test, and regression analysis; Sokal and Rohlf, 1995) were also used when appropriate. Multiple statistical tests across parameters (see Table 1) were controlled for with a false discovery rate correction (Benjamini and Hochberg, 1995).

Descriptive statistics were presented as mean ± standard error of the mean (SE) and range, and *n* denotes the number of individuals and *N* the number of observations from individuals.

Results

In Anatolian ground squirrels, hibernation was characterized by a sequence of multiday torpor bouts, interrupted by brief interbout arousals. Both season and, as we hypothesized, age-sex class had major effects on hibernation patterns (Fig. 2).

Before hibernation, the proportion of individuals exhibiting short torpor bouts varied among age-sex classes (Fisher's exact test, $P = 0.016$). Although most (six of seven) juvenile females exhibited one or two short torpor bouts, only three individuals (one of three adult males, two of nine adult females, and none of four juvenile males) from other age-sex classes exhibited one short torpor bout. All these torpor bouts were observed 1 to 5 days before hibernation. The duration of and minimum Tb during these torpor bouts were 12.1 ± 1.6 hours (4.3–22.3 hours, $n = 9$, $N = 13$) and 25.2 ± 0.6 °C (21.8–27.7 °C, $n = 9$, $N = 13$). However, after hibernation, only one

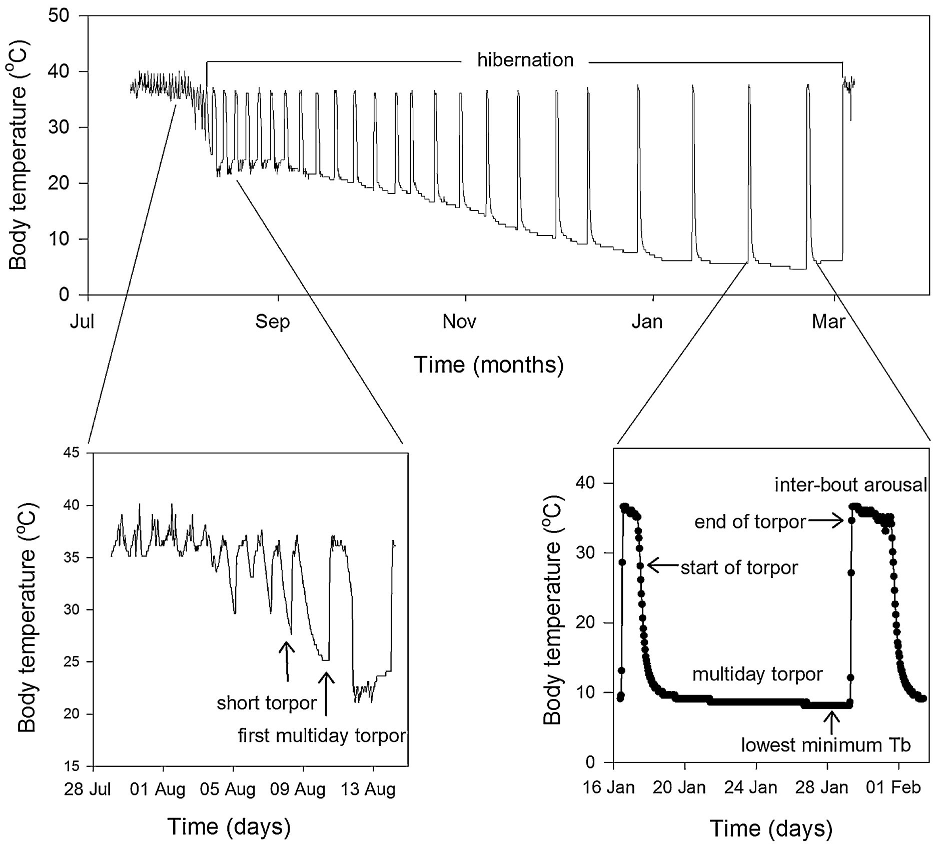

Fig. 1. The details of hibernation and a torpor/interbout arousal cycle under natural conditions. Hibernation is the period from initiation of the first multiday torpor bout to termination of the last multiday torpor bout during which individuals remained continuously underground in the hibernacula.

individual (adult female) exhibited one short torpor bout. This torpor bout was observed 1 day after hibernation. The duration of and minimum Tb during this torpor bout were 1.7 hours and 26.1 °C.

Adult females began hibernation earlier than other age-sex classes. Adults ended hibernation earlier than juveniles in the same sex class, and males earlier than females in the same age class. At the beginning of the active season, adult males were heavier than other age-sex classes (Table 1). When first trapped after emergence from the hibernacula, all adult and most (three of four) juvenile males were reproductive. A non-reproductive juvenile male ended hibernation later than its reproductive counterparts (23 March vs. 04 March ± 1.2 days, one-sample t test, $t_2 = 16.5$, $P = 0.004$). After release to the field, most (three of seven juveniles and eight of nine adults) females were confirmed to reproduce. Males spent less time in hibernation than females (Table 1).

In all age-sex classes, the duration of torpor bouts was short at the beginning of hibernation when Tsoil was at or near its maximum (usually around 19 °C) and increased as Tsoil decreased. The duration of torpor bouts reached its maximum towards the end of hibernation and decreased thereafter when Tsoil was at or near its minimum (usually around 5–6 °C; Fig. 3). Adult males exhibited shorter torpor bouts than other age-sex classes (Table 1). This pattern was also evident especially towards the end of hibernation, i.e. in January ($F_{3,19} = 6.6$, $P = 0.003$) and February ($F_{3,19} = 13.0$, $P < 0.001$; for all other months, $P > 0.05$), when the duration of torpor bouts was averaged by month throughout hibernation (Fig. 3). In all age-sex classes, the duration of interbout arousals also differed throughout hibernation, with patterns generally similar, but inverse, to those described for the duration of torpor bouts. Adults exhibited longer interbout arousals than juveniles in the same sex class, and adult males longer interbout arousals than adult females (Table 1). This pattern was also evident throughout hibernation, when the duration of torpor bouts was averaged by month throughout hibernation (not shown).

In all age-sex classes, the longest torpor bout was observed towards the end of hibernation, i.e. generally in January and February (range of mid-point dates = 29 December–25 February). Thus, all individuals spent less time in hibernation, and exhibited fewer torpor bouts, after than before the longest torpor bout (Fig. 2). The longest torpor bout was of less duration in adult males than in other age-sex classes (Table 1).

Juvenile males exhibited fewer torpor bouts than other age-sex classes. Males spent less time in torpor overall than females, and adult males less time in torpor overall than juvenile males. Adults spent more time in interbout arousal overall than juveniles, and adult males more time in interbout arousal overall than adult females. Regardless of duration of hibernation, adults spent proportionally less time in torpor than juveniles in the same sex class, and adult males proportionally less time in torpor than adult females (Table 1).

Minimum Tb attained during each torpor bout closely followed Tsoil. As expected, the lowest minimum steady-state Tb was also observed towards the end of hibernation when Tsoil was at or near its minimum (Fig. 2). The lowest minimum steady-state Tb attained during hibernation was higher in adult males, but not adult females, than in juveniles (Table 1).

The duration of torpor bouts was negatively correlated with minimum Tb, but the relationship was stronger before than after the longest torpor bout (Fig. 4). Thus, the colder the soil and the colder the ground squirrel, the greater the duration of torpor bouts.

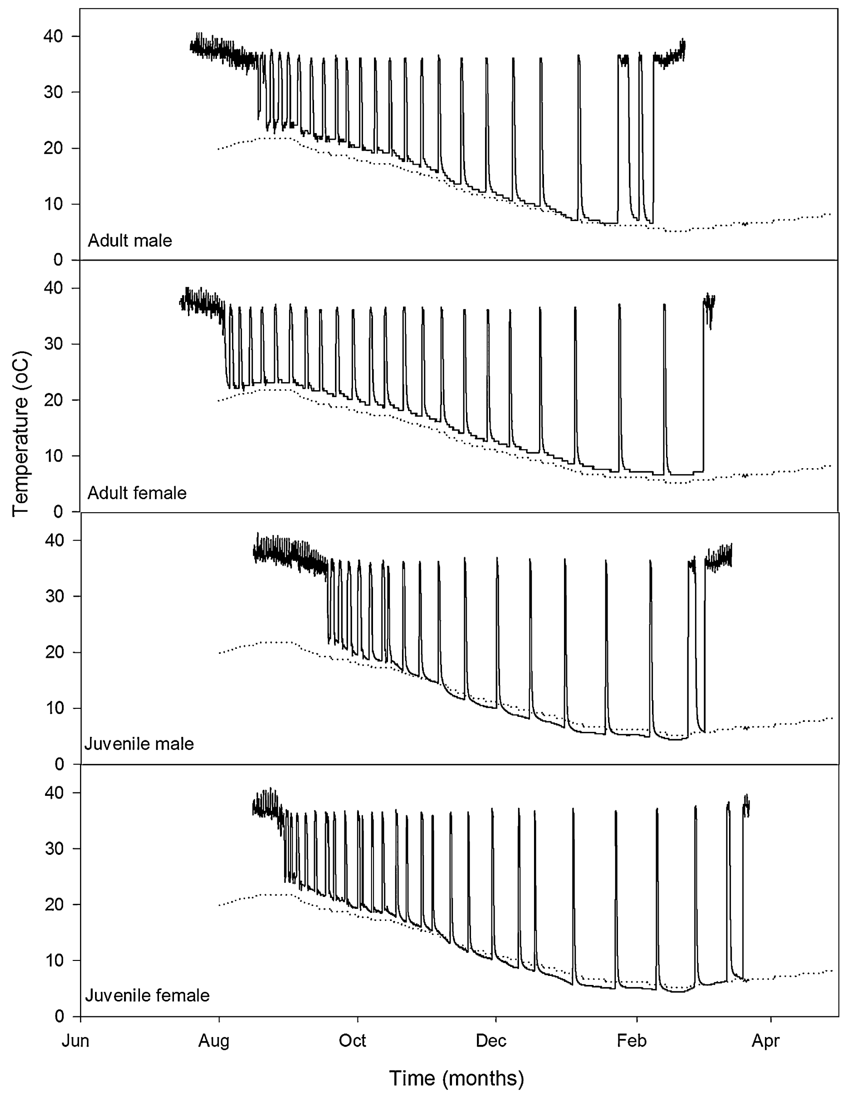

Fig. 2. Body temperature records by month of four representative Anatolian ground squirrels before, duration, and after hibernation under natural conditions. Black lines = body temperature. Dashed lines = soil temperature.

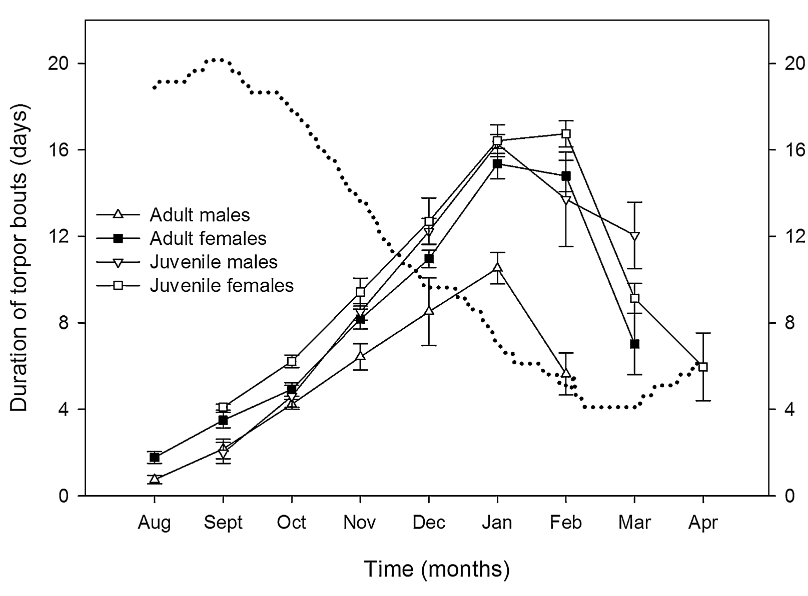

Fig. 3. Mean (±SE) duration of torpor bouts by month of Anatolian ground squirrels throughout hibernation under natural conditions. Each point was based on mean value throughout each month for each individual. Torpor bouts were assigned as a whole to the month in which most of the bout occurred. Dashed line = soil temperature.

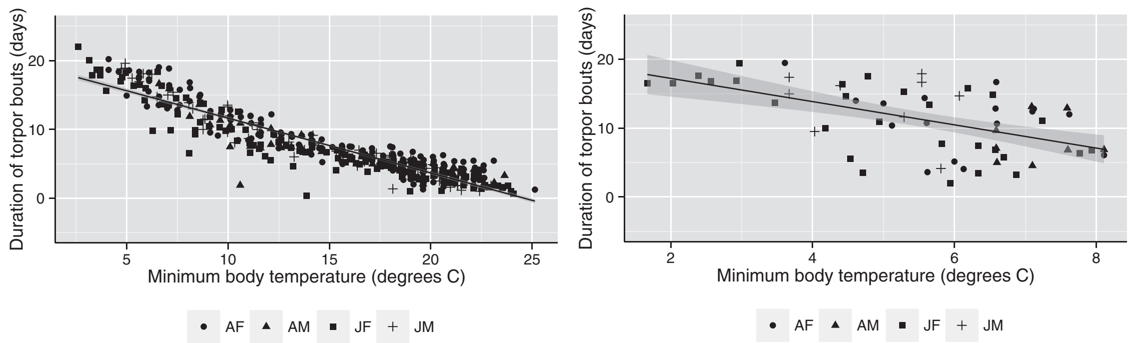

Fig. 4. The relationship of the duration of torpor bouts with minimum body temperature before (left; $R^2 = 0.87$, $P < 0.001$, $n = 23$, $N = 460$) and after (right; $R^2 = 0.29$, $P < 0.001$, $n = 23$, $N = 61$) the longest torpor bout for Anatolian ground squirrels. Adding a second-order (quadratic) term to the models did not improve the overall fit (the results are not presented graphically). Produced using ggplot2 in R (Wickham, 2009).

Discussion

In Anatolian ground squirrels, the first species of Old World ground squirrels to be studied under natural conditions, hibernation was characterized by a sequence of multiday torpor bouts, interrupted by brief interbout arousals. This general pattern of hibernation is universal to all hibernating mammals (Ruf and Geiser, 2014) and has been documented especially well in Holarctic ground squirrels (the tribe Marmotini; Thorington et al., 2012 – most of these are from North America) under both semi-natural (*Urocitellus parryii*, Barnes, 1989; *S. citellus*, Hut et al., 2002) and natural conditions (*U. richardsonii*, Wang, 1973; Michener, 1992; *U. columbianus*, Young, 1990; *Marmota flaviventris*, Florant et al., 2000; *M. marmota*, Ruf and Arnold, 2000; *M. monax*, Zervanos and Salsbury, 2003; Zervanos et al., 2010; *Cynomys parvidens*, Lehmer and Biggins, 2005; *U. parryii*, Buck et al., 2008; Sheriff et al., 2011; *M. broweri*, Lee et al., 2009; *Callospermophilus lateralis*, Healy et al., 2012; *Ictidomys tridecemlineatus*, Kisser and Goodwin, 2012).

Timing of the beginning and end of hibernation based on Tb records generally confirmed patterns of immergence and emergence based on trapping data for this population (Gür and Kart Gür, 2005; Kart Gür and Gür, 2010), although sample sizes are quite small especially for adult males.

Some Anatolian ground squirrels exhibited short torpor bouts (lasting <24 hours) immediately before and after hibernation, as also observed in other species of hibernating ground squirrels (Young, 1990; Michener, 1992; Hut et al., 2002; Zervanos et al., 2010; Healy et al., 2012; Sheriff et al., 2012). The proportion of individuals exhibiting short torpor bouts varied among age-sex classes and between before and after hibernation, with short torpor bouts most often exhibited by juvenile females and before hibernation. However, other individuals (61%) completely bypassed the use of short torpor and immediately initiated multiday torpor bouts, showing that short torpor bouts are not required for initiation of multiday torpor bouts. Some physiological, biochemical, and thermoregulatory adjustments may occur during these short torpor bouts (Strumwasser, 1959, 1960; Russell et al., 2010). However, these short torpor bouts, rather than being preparatory, may also be a continuum of torpor patterns associated with hibernation, because they resemble multiday torpor bouts physiologically and metabolically (Sheriff et al., 2012; Ruf and Geiser, 2014).

In Anatolian ground squirrels, both juveniles and adult males began hibernation later than adult females. Later initiation of hibernation by juveniles compared with adults is typical across hibernating ground squirrels, presumably to allow them to grow and fatten before their first hibernation (Michener, 1984). However, in some species of hibernating ground squirrels (Knopf and Balph, 1977; Fagerstone, 1988; Hut et al., 2002; Sheriff et al., 2011; Healy et al., 2012; for general patterns of immergence, see also Michener, 1984), the function of later initiation of hibernation by adult males compared with adult females remains unclear (Williams et al., 2014). It was speculated that, by remaining active until the end of summer, adult males accumulate and defend a food cache that is subsequently consumed during a pre-emergent euthermic interval, thereby postponing the commencement of dependence on fat stores or assess the locations of the hibernacula used by females (Michener, 1984; Williams et al., 2014). It is important to note here that, in Anatolian ground squirrels, we do not know exact body mass trajectories of adult males towards the end of the active season and whether adult males cache food.

Adult Anatolian ground squirrels ended hibernation earlier than juveniles in the same sex class, and males earlier than females in the same age class. That is, as also observed in many species of hibernating ground squirrels (Michener, 1984), adult males ended hibernation first, i.e. at least about one month earlier than other age-sex classes. This is probably to permit spermatogenesis (reviewed in Williams et al., 2014), which is inhibited at low Tb (Barnes, 1996) and requires at least 12–21 days of sustained euthermia after termination of the last torpor bout in *U. parryii* (Barnes and Ritter, 1993) and *U. richardsonii* (Michener, 1992), because mating occurs shortly after females emerge from the hibernacula (Gür and Kart Gür, 2005; Kart Gür and Gür, 2010). All adult and most juvenile males were reproductive in early spring, but juvenile males had smaller testes and less pigmented scrotum than adult males (observed visually in the field). However, juvenile males ended hibernation later than adult males and at the same time as adult females, but earlier than juvenile females, probably because they are opportunistic in reproduction strategy (Gür and Kart Gür, 2005; Kart Gür and Gür, 2010). The same pattern holds true for *U. elegans* (Fagerstone, 1988). However, for species of hibernating ground squirrels in which juveniles are reproductive after their first hibernation, juveniles normally emerge from the hibernacula (resume aboveground activity) at the same time as adult males (Michener, 1984).

In some species of hibernating ground squirrels (Young, 1990; Michener, 1992; Sheriff et al., 2011), adult males also end hibernation up to several weeks before they emerge from the hibernacula, but this was not observed in the only previous study of Old World ground squirrels (*S. citellus*, Hut et al., 2002). We do not have definite data for Anatolian ground squirrels, but, in the period of the most intensive trapping effort, an adult male was first observed aboveground within 3 days after termination of the last torpor bout.

Anatolian ground squirrels spent about half of the year in hibernation. However, differences in timing of the beginning and end of hibernation resulted in differences in the duration of hibernation among age-sex classes. Adult males spent the least time in

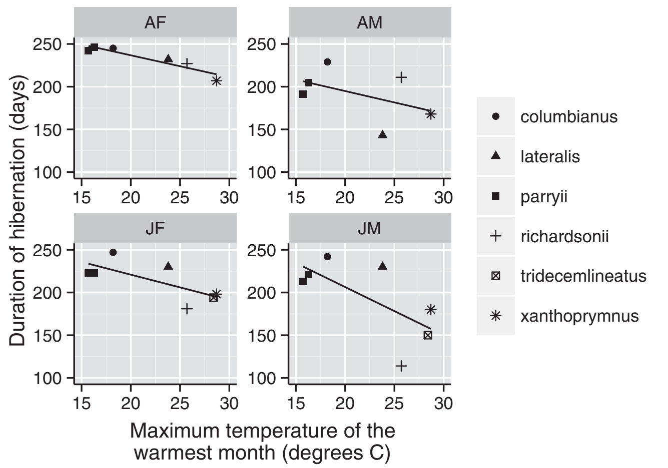

Fig. 5. The relationship of the duration of hibernation with maximum temperature of the warmest month for age-sex classes of the genus *Spermophilus* sensu lato (Helgen et al., 2009) (*U. columbianus*, Young, 1990; *U. richardsonii*, Michener, 1992; *U. parryii* at Toolik Lake and Atigun river, Sheriff et al., 2011; *C. lateralis*, Healy et al., 2012; *I. tridecemlineatus*, Kisser and Goodwin, 2012; *S. xanthoprymnus*, this study). Maximum temperature of the warmest month was obtained from the WorldClim-Global Climate Data (www.worldclim.org/current) for the areas in which species were studied. Adult females (AF): $R^2 = 0.86$, $P = 0.007$, $n = 6$; Adult males (AM): $R^2 = 0.21$, $P = 0.356$, $n = 6$; Juvenile females (JF): $R^2 = 0.51$, $P = 0.070$, $n = 7$; Juvenile males (JM): $R^2 = 0.45$, $P = 0.098$, $n = 7$. Produced using ggplot2 in R (Wickham, 2009).

hibernation (5.5 months), followed by juvenile males (6 months), juvenile females (6.5 months), and then adult females (7 months). The duration of hibernation differs among species of hibernating ground squirrels and the magnitude of this difference varies across age-sex classes; these may be partly explained by climate and that age-sex classes respond differently to climate, respectively (Fig. 5).

In Anatolian ground squirrels, the duration of torpor bouts was short at the beginning of hibernation, increased as Tsoil decreased, reached its maximum toward the end of hibernation, and decreased thereafter. Malan (2010, 2012) suggested that, in Anatolian ground squirrels (data from Kart Gür et al., 2009), the duration of torpor bouts is controlled by a temperature-dependent circadian (i.e. the torpor-arousal) clock, as also in *U. parryii* and *S. citellus* (data from Barnes and Ritter, 1993 and Hut et al., 2002, respectively). This clock gives the animal a safe mechanism to elicit interbout arousals in due course. It also presents another selective advantage: by a passive thermodynamic effect, it will expand the duration of torpor bouts when ambient temperature and Tb decrease (Malan, 2010, 2012). Accordingly, in Anatolian ground squirrels, the duration of torpor bouts varied inversely with minimum Tb, and minimum Tb attained during each torpor bout closely followed Tsoil. The same pattern holds true for other species of hibernating ground squirrels (Young, 1990; Michener, 1992; Hut et al., 2002; Healy et al., 2012; Kisser and Goodwin, 2012). However, Ruf and Geiser (2014) discussed that a metabolism-dependent, so-called hourglass mechanism may control torpor bout/interbout arousal cycles and this seems a more parsimonious explanation than the assumption of a temperature-dependent circadian clock, although these two mechanisms may not be mutually exclusive. In terms of energy savings, it is believed that it is of greater benefit for hibernating mammals to increase the time spent in torpor as ambient temperature decreases, because energy stores, e.g. in the form of body fat, are limited (Humphries et al., 2003).

Adult male Anatolian ground squirrels exhibited hibernation characteristics rather different from those of other age-sex classes, spending less time in hibernation, exhibiting shorter torpor bouts and longer interbout arousals, especially toward the end of hibernation, and spending proportionally less time in torpor, as also generally observed in many species of hibernating ground squirrels (Buck et al., 2008; Young, 1990; Michener, 1992; Healy et al., 2012). These differences may reflect trade-offs between the energy-saving benefits of torpor and the reproductive benefits incurred by early ending of hibernation (Healy et al., 2012). Because adult male Anatolian ground squirrels remain euthermic longer during winter, especially during the late winter when Tsoil at the expected depth of the hibernacula is at or near its lowest value and food is still scarce, they probably have higher risk of starving. Thus, adult males are larger and store more fat and therefore have greater overwinter fasting endurance, which may enable them to survive overwinter and reproduce the following spring (Gür, 2010), as is the case, for example, in *U. richardsonii* (Gür and Kart Gür, 2012).

In Anatolian ground squirrels, minimum Tb attained during each torpor bout closely followed Tsoil (recorded at the expected depth of the hibernacula in one location close to most burrows). However, since the location of the data logger recording Tsoil was not exactly the same as the different hibernacula and/or the insulative quality (temperature regime) of the hibernacula might be different, minimum Tb was sometimes slightly below (in juveniles) or above (in adults) Tsoil. Moreover, the lowest minimum steady-state Tb observed towards the end of hibernation when Tsoil was at or near its minimum ranged from 1.7 to 6.2 °C (mean ± SE, 4 ± 0.4 °C) in juveniles and from 3.6 to 8.1 °C (mean ± SE, 5.8 ± 0.4 °C) in adults. These may indicate that adults have used deeper and/or more insulated hibernacula than juveniles. Anatolian ground squirrels did not display zero or subzero core Tb in Central Anatolia, although some species of hibernating ground squirrels physiologically tolerate core Tb below 0 °C during torpor bouts (Table 2 in Healy et al., 2012; Table 1 in Ruf and Geiser, 2014). However, we cannot exclude the possibility that Anatolian ground squirrels can physiologically tolerate zero or subzero core Tb in colder, more seasonal environments in Anatolia (e.g. northeastern Anatolia), where Tsoil drops to ≤0 °C (Atalay and Mortan, 2006).

In conclusion, we observed that hibernation patterns in free-living Anatolian ground squirrels were influenced by age-sex classes, as well as environmental conditions. However, populations of the same species living in different environments may vary in regard to hibernation characteristics and therefore research on a single population may lead to incomplete understanding of how hibernating mammals respond to the climatic and ecological conditions of the environment in which they live (Stawski, 2012). Thus, Anatolian ground squirrels, whose range encompasses highly different climatic and ecological conditions (Kart Gür and Gür 2010; Gür, 2013), should be studied similarly in colder, more seasonal environments (e.g. northeastern Anatolia). This may also be critical to predicting the consequences of ongoing and future global climate changes on hibernating mammals and therefore help guide management initiatives.

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