

Sticking Together: Energetic Consequences of Huddling Behavior in Hibernating Juvenile Garden Dormice*

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ABSTRACT

Hibernation, or multiday torpor, allows individuals to save energy via substantial reductions of metabolism and body temperature but is regularly interrupted by euthermic phases called arousals. Social thermoregulation, or “huddling,” can act in synergy with torpor in reducing individuals’ energy and heat losses. In the wild, the garden dormouse (*Eliomys quercinus*) combines both strategies, which are crucial for winter survival of juveniles with limited prehibernation body fat reserves. We investigated via thermographic and temperature measurements (i) the energetic impact of huddling during an arousal from deep torpor, (ii) the dynamics of huddling behavior during hibernation, and (iii) its consequences during the entire winter in juvenile garden dormice. Thermographic images revealed a significant effect of huddling on

torpor energetics, as it reduced heat exchange and mass loss by two-thirds in huddling versus single individuals during arousal. Our investigation of the dynamics of huddling further revealed a “random-like mechanistic” behavior during winter hibernation, as arousals from torpor were not always initiated by the same individuals. Animals took turns in initiating rewarming within a group, and the individual with highest body temperature during arousal entered into torpor later than the others within the huddle. The animals share both costs and benefits of huddling during arousals, without any energetic benefit of huddling over the entire winter on an individual level. We conclude that the dynamics of social thermoregulation during hibernation seems to counterbalance its benefit of reducing energetic costs associated against the energy-demanding process of rewarming from torpor.

Keywords: social thermoregulation, torpor, cooperative behavior, body temperature, metabolic rate, energy saving.

Introduction

Surviving periods of food shortage constitutes a real challenge for small birds and mammals, especially for juveniles struggling to sustain both growth and development during a time of major energy losses and inexperience in foraging (Geiser 2008; Geiser et al. 2019). To face environmental reduction of energy resources, animal species commonly use several adaptive physiological and behavioral responses. Among those, the use of energy-saving strategies, such as hypometabolism (i.e., torpor and social thermoregulation, or “huddling”), is likely to provide many benefits to young individuals (Heldmaier and Ruf 1992; Geiser and Ruf 1995; Gilbert et al. 2010; Ruf and Geiser 2015).

Torpor is a highly efficient energy-saving strategy involving an active and controlled reduction of metabolic rate (MR) followed by a decrease of body temperature (T_b), enabling individuals to survive energetic bottlenecks (Lyman et al. 1982). To date, 214 heterothermic species have been identified and can be categorized as daily heterotherms, species showing rather shallow bouts of torpor lasting less than 24 h, or as hibernators that undergo long and deep torpor bouts of a few days to weeks interspaced by periodic phases of rewarming (for review, see Ruf and Geiser 2015). In daily heterotherms, mean minimum

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torpid MR (TMR) is ~19% of basal rates, and T_b usually reaches values between 12°C and 25°C during torpor (for review, see Ruf and Geiser 2015). During hibernation, animals reach minimum TMR of ~4% of basal rates, and their T_b decreases along ambient temperature (T_a) until minimum values are reached, which are on average between 0°C and 10°C for most hibernators (e.g., Buck and Barnes 2000; Heldmaier et al. 2004). During hibernation, periodic arousals, which are necessary for the reactivation of some major physiological functions such as immune and cardiovascular systems, are associated with an extreme increase in metabolic activity (~8–10 times greater than individual basal levels). However, arousals are also extremely costly. In particular, periodic arousals account for the majority of energetic costs during hibernation (i.e., ~70%–80% of energy expended by individuals during the entire winter; Boyer and Barnes 1999). Moreover, an upregulation of MR can cause oxidative damage via increased oxidative stress level. For instance, oxidative stress can contribute to the shortening of telomeres (Von Zglinicki 2002), which has been reported to occur in both hibernating edible dormice and garden dormice during winter (Hoelzl et al. 2016; Nowack et al. 2019). Nevertheless, the use of torpor is thought to provide great benefits for juveniles, such as an increase in survival rate during winter via a reduction of metabolism, locomotor activity, and energy loss (Geiser and Brigham 2012), or by helping individuals to fatten before hibernation—as previously demonstrated in garden dormice—to compensate for reduced food availability or a late start to the breeding season (Giroud et al. 2014; Mahrlet et al. 2018).

An alternative strategy used by several small animal species to substantially reduce their energy requirements, without entering into a hypometabolic state, is the use of social thermoregulation, or huddling. More than 67 species of mammals and 25 species of birds employ this strategy, which has the major advantage that it can be combined with other behaviors (for review, see Gilbert et al. 2010). Contrary to torpor, this cooperative group behavior reduces the exposure of the body surface to cold and is characterized by a close aggregation of individuals, lowering heat loss and energy expenditure compared with isolated individuals (for review, see Gilbert et al. 2010). The use of huddling by individuals is expected to provide many benefits required for growth (for review, see Gilbert et al. 2010), such as the maintenance of a high T_b and an increase of the temperature in the nest (T_{nest}), that would be particularly advantageous for juveniles threatened by extreme heat loss (Nowack and Geiser 2016). Huddling behavior reduces energy expenditure by 6%–53% depending on T_a , density of aggregations, animal species, and number of individuals involved in the huddle (for review, see Gilbert et al. 2010).

Interestingly, the behavior of huddling can act in synergy with the use of torpor, as it enhances energy saving by both increasing the benefits and reducing the costs associated with torpor. In particular, the garden dormouse (*Eliomys quercinus*, Gliridae) is known to use both strategies of huddling and torpor under natural conditions (for review, see Gilbert et al. 2010). Several studies reported positive energetic benefits of huddling with longer torpor bouts and more energy saving for individuals kept in groups than individually (Jefimow et al. 2011; Nowack and Geiser 2016). Also, the use of torpor is associated with food deprivation and body mass

(BM) loss, which can be buffered by the use of huddling (Arnold 1988). This is of particular relevance for juveniles, as they do not always have a significant level of fat stores before their first winter. During hibernation, most of the thermal benefits of huddling result from decreased heat loss and energy expenditure during rewarming and euthermic phases (Boyles et al. 2008). However, the use of huddling does not always appear to be beneficial, as some deleterious effects from group housing might hinder the energetic benefits of social thermoregulation (Ruf and Arnold 2000; Dausmann and Glos 2014; Nowack and Geiser 2016). Notably, it appears that the use of social thermoregulation and potential associated energy savings depend on environmental conditions and group composition, triggering asymmetric costs and benefits for individuals with different BM (i.e., variable amounts of energy reserves; Gębczyński 1969; Gębczynska and Gębczyński 1971). Nevertheless, small individuals are likely to benefit more than large animals from energy savings owing to passive rewarming and lower heat loss during the huddling behavior, which might hence be advantageous for juveniles with low levels of fat reserves. In particular, juvenile garden dormice that are born late (from late August to September) within the reproductive season, face a particular challenge with a much-reduced time before the start of winter to grow and develop enough fat reserves, a strong predictor of winter survival and future reproductive success (Moreno 1988; Lenihan and van Vuren 1996; Giroud et al. 2012, 2014; Mahrlet et al. 2018).

In this study we aimed at determining the effects of huddling on torpor behavior in late-born hibernating juvenile garden dormice during winter. Specifically, we investigated via thermographic and core T_b measurements (i) the energetic impact of huddling during arousal from deep torpor, (ii) the dynamics of huddling behavior during hibernation, and (iii) its consequences during the entire winter in male and female juvenile dormice. We hypothesized that huddling would provide energetic benefits to juveniles, considering the entire hibernation season, notably via minimizing energetic costs associated with periodic arousals from torpor. We also hypothesized that arousals within the huddle will be systematically initiated by the individual or individuals with the largest amount of body fat reserves (i.e., the biggest individuals).

Material and Methods

Ethics Statement

All experiments carried out in this study were approved by the institutional ethics committee and the national Austrian authority in accordance with the Austrian Animal Experimentation Act, Tierversuchsgesetz 2012 (BMBWF-68.205/0175-V/3b/2018).

Animals

The garden dormouse (*Eliomys quercinus*) is a small hibernator inhabiting western and central Europe, with some scattered populations in eastern European regions. This rodent shows different hibernation patterns depending on its distribution range and its habitat (Mrosovsky and Lang 1980). According to Moreno (1988)

and Gil-Delgado et al. (2006), hibernation of garden dormice may last from 1 mo (southern part of Europe) to about half a year (northern part of Europe). Garden dormice are known to use both strategies of huddling and torpor, notably during winter (for review, see Gilbert et al. 2010).

All animals included in the experiments derived from a stock originally captured in western Germany (Ebertseifen, Niedersfischbach, Germany), which is maintained in captive conditions under natural fluctuations of photoperiod and T_a at the Research Institute of Wildlife Ecology (University of Veterinary Medicine Vienna, Vienna, Austria; 48°15'N, 16°22'E). Animals were individually marked with miniature subcutaneous transponders (TierChip Dasmann, Animal ID ISO 11784/85 FDX-B Standard, Tecklenburg, Germany; <https://tierchip.de/>) for identification. Before winter, garden dormice prepare themselves for hibernation by fattening and lowering their metabolism. During winter, animals were placed in fridges kept at a stable T_a of 3.53°C \pm 0.45°C (credible interval [CI]: 2.63°C–4.43°C) and under constant darkness. Females enter into hibernation 2 wk earlier than males, and juveniles start hibernation after adult individuals (Mrosovsky and Lang 1980; Moreno 1988). Furthermore, late-born juvenile garden dormice enter hibernation around 4 wk after early-born individuals, as previously described (Stumpfel et al. 2017; Mähler et al. 2018). In the experiment, winter hibernation lasted 16 wk for late-born females and 14 wk for late-born males; both sexes emerged from hibernation at the same time by mid-March, similar to garden dormice kept under natural fluctuations of T_a and photoperiod (Stumpfel et al. 2017; Mähler et al. 2018).

Experimental Design

Experiments were conducted over two consecutive years (2018–2019 and 2019–2020) from September 24, 2018, to March 14, 2019, and from September 24, 2019, to March 18, 2020. In 2018–

2019, all individuals were born late (August 7–20, 2018), whereas in 2019–2020, animals were born either early (May 13–22, 2019) or late (August 11–23, 2019) in the reproductive season. Over both winters, a total of 66 juvenile garden dormice (2018–2019: 10 late-born males and 18 females; 2019–2020: 27 early-born males, 5 late-born males, and 6 late-born females) were enrolled in one or more of the four conducted experiments (for specific details, see tables 1, A1). Some individuals were provided with food ad lib. and others intermittently fasted (for more details, see tables 1, A1). In short, the experiments included (i) the assessment of hibernation patterns and BM loss in 31 late-born juveniles, (ii) the recording of MR during winter hibernation in a subset of 12 late-born individuals, (iii) the study of the dynamics of huddling (i.e., the rank of arousal and cooling within a group) using T_b recordings (from 7 to 13 torpor-arousal cycles) during winter hibernation in a subset of 20 late-born juveniles kept in groups of four, and (iv) the use of a mechanistic approach to investigate the energetic impact of huddling during a single arousal in the 27 early-born individuals. In this latter experiment, carried out February 3–7, 2020, we specifically used male juveniles born early in the reproductive season because of greater between-individual differences in BM before and during hibernation (CI: 92.3–115.3 g) compared with those reported in late-born individuals (CI: 85.3–98.1 g; Stumpfel et al. 2017).

During winter, animals were kept individually or grouped by four males or females (for summary, see tables 1, A1), which represents the optimal group size to maximize energetic benefit (Gilbert et al. 2010). To follow individual hibernating patterns, all of the animals were implanted with small temperature data loggers that monitored core T_b every 4 min (see below for details). A subset of individuals or groups (see table 1 for sample sizes) were placed in respirometry cages for MR recordings (see below for details) to assess metabolic costs in single and grouped individuals, as well as to estimate the energy expenditure related to interbout arousals. T_{nest} 's were further recorded through a nest

Table 1: Experimental groups included in the study

Experiment	Description	Year(s)	Size
Hibernation pattern and body mass loss	Comparison of body mass loss and torpor parameters (total and mean torpor bout durations, torpor frequency) in single vs. grouped fasted LB male and female juveniles	2018–2019, 2019–2020	$n = 31$ (15 M, 16 F); 15 singles (7 M, 8 F); 4 groups (8 M, 8 F)
Metabolic rate	Comparison of metabolic rate during torpor and euthermia in single vs. grouped fasted LB male or female juveniles	2018–2019	$n = 12$ (6 M, 6 F); 4 singles (2 M, 2 F); 2 groups (4 M, 4 F)
Dynamics inside a group	Determination of the proportion to rewarm first inside a group of LB male or female juveniles; investigation of the rank of arousal and the rank of cooling in grouped LB male or female juveniles	2018–2019	$n = 20$ (4 M, 16 F); 5 groups (4 fast M, 8 fast F, 8 ad lib. F)
Isolated arousal	Comparison of body mass loss after an isolated arousal in single vs. grouped EB male juveniles; estimation of heat exchanges in single vs. grouped EB male juveniles	2019–2020	$n = 27$; 4 groups (16 M); 11 singles (M)

Note. Groups are unisex, male (M), or female (F), and individuals were either early born (EB) or late born (LB) in the reproductive season. For the dynamics inside a group experiment, individuals were hibernating without food (fast) or with food ad lib.

logger equipped with a connection outside the cooling unit to collect the data without disturbing the animals, as used in previous studies (Giroud et al. 2014; Nowack et al. 2019).

During the experiments to investigate the energetic effect of huddling on the costs of arousal, rewarming from torpor was triggered in single or grouped animals in a climate chamber (TPK600, Feutron, Langenwetzendorf, Germany) maintained at constant T_a ($7.3^\circ\text{C} \pm 0.8^\circ\text{C}$), with a relative humidity of $68.9\% \pm 5.9\%$. T_a was measured every minute by an external logger located next to the animals (EL-USB-2, Lascar Electronics, Whiteparish, UK; resolution: 0.58°C ; accuracy: $\pm 0.58^\circ\text{C}$), and relative humidity was measured by a sensor located on the wall inside the climate chamber. We used T_{nest} data to determine whether animals were euthermic or torpid. Values above 4°C by several degrees indicated that the animal or group was euthermic and exhibiting an arousal (for details on the method of T_{nest} measurements, see fig. S1 in Giroud et al. 2014). This was required to determine which animal or group should be selected to study the arousal from torpor (see experiment “isolated arousal” in table 1). The length of arousal was recorded, and thermographic images of the animals were assessed (see below for details). From the recordings of the other data, the analysis of isolated arousals was divided into two parts: one for surface temperature (T_s) below 8°C and one for T_s above 8°C , corresponding approximately to T_a in the climate chamber.

Surgical Implantations of Loggers and Temperature Measurements

All individuals ($n = 66$) were surgically implanted with small temperature loggers (1.7 g , 1.1 cm^3 , custom made and calibrated at our institute; storage capacity: 100,000 temperature readings; accuracy: $\pm 0.1^\circ\text{C}$), as previously described (Giroud et al. 2018). All loggers were surgically removed from the animals after hibernation. For more details, see “Surgical Implantations of Loggers and Temperature Measurements” in the appendix. Unfortunately, because of a technical problem with the batteries, most of the implanted temperature loggers for early-born males failed, leading to a major loss of $\sim 85\%$ of temperature data (data from only four of 27 loggers were retrieved).

Hibernation Pattern and Body Mass Loss

Core T_b recorded during winter hibernation was used to compute the hibernation length, arousal frequency, mean of separate interbout euthermia durations (EBDs) and sum of EBDs (total EBD), mean of separate torpor bout durations (TBDs) and sum of TBDs (total TBD), and minimum T_b during torpor (torpid T_b). During hibernation, each animal was considered torpid if its T_b was below 18°C . Some of the torpor frequencies were completed using the T_{nest} recording using a threshold of 5°C to determine whether the animal was considered torpid (below 5°C) or euthermic (above 5°C).

Using temperature data during winter, we focused on groups to investigate the dynamics of huddling, especially the order of animals arousing from torpor (i.e., arousal rank) or cooling down

after arousal (i.e., cooling rank) within a group (1 for the first individual and 2, 3, and 4 for the following ones). To do so, we looked at torpor and euthermia parameters for each arousal/cooling (between 7 and 13 cycles, depending on groups). We used single EBD or total EBD, TBD, maximum and mean temperatures during euthermia (max T_b and mean T_b), and torpid T_b . We tested the effects of euthermia and torpor parameters that preceded the rewarming to study the rank of arousal (euthermia $_{n-1}$, cooling $_{n-1}$, torpor $_{n-1}$ for the n th arousal). To investigate variables influencing the cooling rank, we used torpid and euthermic parameters before the cooling episode (torpor $_{n-1}$, arousal $_{n-1}$, euthermia $_{n-1}$ for the n th cooling).

To investigate BM loss during winter, all of the individuals from 2018–2019 and 2019–2020 were weighed before hibernation (prehibernation BM) to the nearest of 0.1 g by using a balance (Mettler Toledo, PM34, Delta Range). BM was again measured to the nearest 0.1 g in March after the entire period of hibernation. In February 2020, during the experiment focusing on isolated arousals including 27 early-born males (4 groups of 4 individuals and 11 isolated individuals), BM before (prearousal BM) and after an isolated arousal were determined for each animal to the nearest 0.01 g using a precision balance (Aroma-Zone, PM500, Delta Range). Males and females showed different prehibernation BM, but in both sexes, individual or grouped animals had similar BM at the beginning of the experiment (see table A2). For the isolated arousal experiments, all early-born males from the two housing conditions had similar prearousal BM in February (single vs. groups: $94.1 \pm 3.4\text{ g}$ vs. $91.3 \pm 2.1\text{ g}$; $t = -0.68$, $P = 0.5$).

Respirometry for Measurements of Metabolic Rate

Oxygen consumption ($\dot{V}\text{O}_2$) was recorded using an open-flow respirometry system, as described previously (Nowack et al. 2019). Six cages were recorded at the same time, between November 2019 and March 2020. For more details, see “Respirometry for Measurements of Metabolic Rate” in the appendix.

Mass-specific MR was computed and expressed separately as mean TMR, maximum euthermic MR (max EMR, calculated as the mean of the five highest MR values), and mean EMR. To estimate the energetic cost of arousals during hibernation, we first computed the average cost of an arousal by summing $\dot{V}\text{O}_2$ during rewarming and subsequent euthermia (i.e., when $\dot{V}\text{O}_2$ started to increase until it decreased before the next torpor bout). Then we estimated the total cost of a torpor-arousal cycle by calculating the average cost of an arousal episode and subsequent euthermia and the average metabolic cost of torpor, multiplied by the average duration of arousal or of torpor bout, respectively. Then we calculated the relative cost of arousal and subsequent euthermia compared with the cost of a complete torpor-arousal cycle, as previously described in Karpovich et al. (2009).

Thermography for Assessments of Surface Temperature and Heat Exchange

In February 2020, one period of arousal ($n = 1$) was recorded using a thermovision camera (FLIR T520, FLIR System; <https://>

www.flir.fr) in a walk-in climate chamber. An isolated arousal was recorded per group (four groups of four individuals) or individual (11 single individuals). Pictures were taken every minute to assess the T_s of the animals during the arousal (fig. S1). After quick removal from the cooling unit, animals were weighed and placed in the climate chamber in an artificial nest facing the camera to allow individuals to keep the same posture as during hibernation in the cooling unit (fig. 1). A chronometer was started when the first picture was taken, after a short stimulation of the paws with a metal clamp to induce the arousal with reduced human contact and avoid emotional rewarming. The last photo was taken when the animal was changing its posture or leaving the nest (Phillips and Heath 2004; Hodges et al. 2008).

We compared pictures with a lateral view or dorsal view (fig. 1) of the animal to standardize the position of the body axes in relation to the thermovision camera (Tattersall 2016; Ferretti et al. 2019). T_s for the body area was estimated using the ThermoCAM Researcher version 2.10 software (FLIR system). To get the T_s for each individual and the entire group, we drew areas around each body and another one including the four individuals (see fig. 1). As the climate chamber had a temperature of 8°C, the analysis of isolated arousals was divided into two parts—one for

T_s below 8°C and one for T_s above 8°C—to separate passive/active warming by the environment. Heat exchange was calculated using the Thermimage package in R (Tattersall 2019), assuming an emissivity of 0.96 and incorporating other parameters such as T_a , ground temperature, relative humidity (from the climate chamber), distance from the object (given by the thermovision camera), and the length of each animal. For individually hibernating animals, T_a was recorded from an external logger placed on the table in the climate chamber (also used as the ground temperature in the calculations). For grouped individuals, T_a was approximated using T_a recorded with the external logger at the beginning of the arousal. However, because of local rewarming, when the group temperature was greater than that recorded with the logger (8°C), we used the group temperature as the local T_a in the calculation.

Statistical Analysis

Statistical analyses were conducted using R (ver. 3.5.1; R Core Team 2018). Because of group housing (individually or grouped), we accounted for the existence of interdependency between individuals by including cage ID as a random effect in all statistical models. For each variable, we used a model selection based on Akaike's information criterion corrected for small sample size (AICc; Akaike 1973). Normality of residuals was assessed by inspecting quantile-quantile plots for linear model, and overdispersion was checked for generalized linear mixed effects models with Poisson distribution.

Entire Winter Study. Linear mixed effects models were used to study the impact of social thermoregulation and sex on hibernating parameters and BM loss over winter. Specifically, we tested the effects of housing condition and sex (and their interaction) on BM, total TBD, total EBD, and arousal frequency during winter hibernation. As animals were studied during different years, we further used the term year as a random factor, and because of differences in hibernation duration, the length of winter hibernation period (hib duration) was included as a fixed variable. We further tested the effect of housing condition, sex (and their interactions), and T_{nest} on MR parameters and torpid T_b using linear mixed effects models, including the terms BM and cage ID as random factors. We applied a type II ANOVA (when only main effects were included) or type III (in case of interactive terms) to test significance on variables from the best model (Anova in library car; Fox and Weisberg 2011).

Dynamics Inside a Group. Generalized linear mixed effects models with a Poisson distribution were used to test effects of torpor parameters (EBD, TBD, max T_b , mean T_b , and torpid T_b), sex, and prehibernation BM on the order of animals arousing from torpor (i.e., arousal rank) or cooling down after arousal (i.e., cooling rank) within a group for every torpor-arousal cycle. As preliminary studies showed that food treatment had no effect, we did not include it in the model selection. Cage ID was used as a random factor, and the length of winter hibernation period (hib duration) was included as a fixed variable. As many variables were

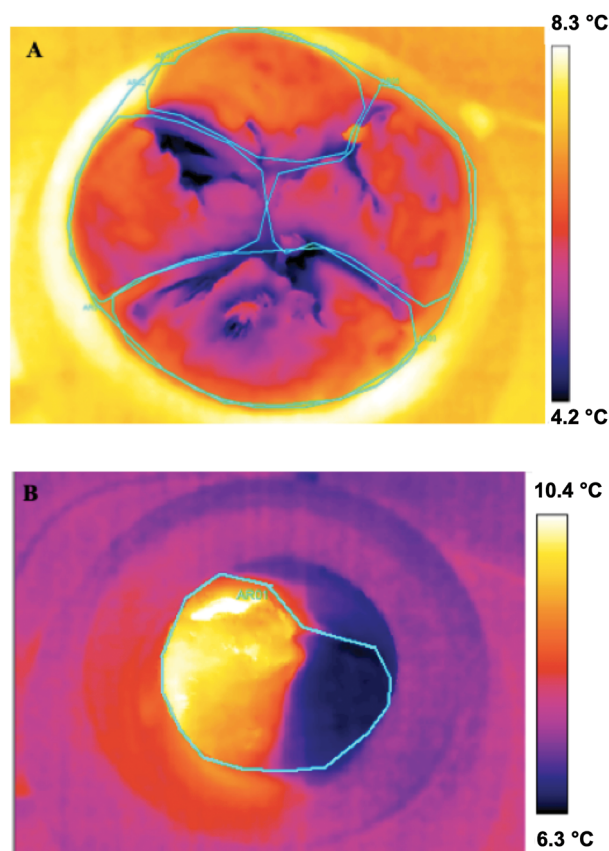


Figure 1. Thermal images of a group of four individuals (A) and a single individual (B) during one standardized arousal from deep torpor. The blue line limits the areas used to measure the surface temperatures using the software ThermoCAM Researcher version 2.10 from FLIR system.

included in the models, we used the variance inflation factor (Naimi et al. 2014) to test for possible collinearity, and when needed, we reduced the number of variables. This was the case for max T_b , which was excluded from the model because of high correlation with mean T_b . To study the proportion of rewarming as the first animal in a group, we used linear models to test for the effect of torpor parameters (total EBD, total TBD, mean T_b , and torpid T_b), prehibernation BM, BM loss, and sex on this variable. As above, the length of winter hibernation period (hib duration) was included as a fixed variable in the model. To test the variables for significance, we performed a type II ANOVA on the variables of best models (lme in library nlme [Pinheiro et al. 2018]; dredge function in library MuMIn [Barton 2018]; Anova in library car).

Investigations of Single Arousals. We used linear mixed effects models with the term cage ID as a random factor to test the effect of housing on BM loss (i.e., postarousal BM corrected for prearousal BM, which was included in the model). We included the last T_s (T_s end) that we measured with the thermovision camera as a fixed variable in the model to take into account the differences in arousal length between individuals (recording lasted until the first animal to move). To investigate heat exchange, we used linear mixed effects models with the term cage ID as a random factor to study the difference between groups (heat exchange of the entire group) and single individuals. We also used individual ID to compare all individuals (heat exchange of each animal, even inside a group). In the model constructed to reveal potential links between heat exchange and the rank of arousal, focusing on groups, we also used individual ID as a random factor. In each model, we tested for the effect of prearousal BM, BM loss, and housing condition. Finally, we performed a type II ANOVA on the best model (lme in library nlme [Pinheiro et al. 2018]; dredge function in library MuMIn [Barton 2018]; Anova in library car) to test the variables for significance.

Results

Energetic Benefits of Huddling during Single Arousals

Heat Exchange. For the first part of arousal (i.e., T_s below 8°C), we did not find any effect of housing condition (group vs. single) and BM variation on heat exchange, as all individuals rewarmed passively (positive heat exchanges; fig. 2A). For the second part of arousal (i.e., T_s above 8°C), we found an effect of housing condition (group vs. single; fig. 2B; tables 2, 3). Individuals kept in groups showed higher and positive heat exchange compared with single individuals for which heat exchange became negative, indicating that single individuals actively rewarmed, while most of the grouped animals overall benefited from passive rewarming. Then inside a group, heat exchange differed depending on the rank only during the second part of arousal (fig. 3; tables 2, 3). The first individual to arouse rewarmed actively (heat exchange is null), while the others benefited from passive rewarming, as indicated by positive heat exchanges (fig. 3).

Body Mass Loss. Grouped dormice showed a 2.5-fold lower BM loss compared with animals rewarming alone (fig. 4) during an isolated arousal (tables 2, 3).

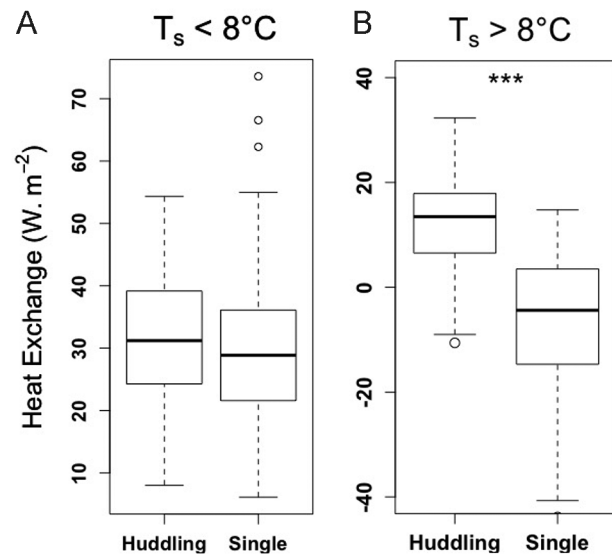


Figure 2. Boxplots of environmental heat exchanges (W/m^2) of individuals housed singly (single) or in groups of four (huddling) either during passive heating at surface temperature (T_s) less than 8°C (A) or during active rewarming at T_s greater than 8°C (B) during one standardized arousal. The temperature of the climate chamber in which the experiments took place was set to 8°C . $n = 27$ individuals: 11 single and 16 grouped individuals. *** $P < 0.001$.

Dynamics Inside a Huddle

Arousals ($n = 10 \pm 3$) were not always initiated by the same individual, and one animal never initiated two arousals in consecutive bouts. In fact, the frequency with which an individual initiated an arousal was of 0.25 ± 0.14 (CI: 0.08–0.54), indicating that each animal within the huddle initiated rewarming once during the hibernation period. We found no implications and/or no effects of prehibernation BM, BM loss, or torpor parameters on the proportion to initiate an arousal (tables 4, 5) or the rank of arousal (table 5). However, these variables were included in the best model (table 4), suggesting a relative importance of these variables in explaining the dynamics of huddling.

Energetic Benefits of Huddling during Winter Hibernation

Hibernating Patterns and Body Mass Loss over Winter. Total TBD differed according to the housing condition (i.e., group; tables 6, 7). Animals hibernating in groups show a reduced total TBD compared with single individuals (table A3). Although housing condition (group) and sex appeared in the first models for total EBD and arousal frequency, those effects were not different (tables 6, 7, A3). Finally, we found no effect of housing condition on BM loss over winter hibernation (table A2).

Metabolic Rate and Temperature in Torpor. Arousals represented an estimated 75.8% of energy expenditure during hibernation, with no differences between grouped and individual animals (single vs. groups: $73.1\% \pm 15.8\%$ vs. $77.4\% \pm 15.3\%$; $t = 1.5$,

Table 2: Best candidate models ($\Delta AICc < 2$) explaining heat exchanges and body mass change during the isolated arousal experiments

Parameter, test, model	AICc	ΔAIC	Weight
Heat exchange:			
Group vs. single:			
Group	2,926.5	.00	.53
Group + prehibernation BM	2,928.5	1.93	.20
Within a group:			
Rank	1,252.6	.00	.39
Rank + BM loss	1,252.9	.34	.33
BM:			
Group vs. single:			
Group + prearousal BM + T_s end	-109.8	.00	.76

Note. Explanatory variables were housing condition (group), surface temperature at the end of arousal (T_s end), rank of arousal (rank), body mass before arousal (prearousal BM), and body mass loss (BM loss). For heat exchanges, we investigated the effect between grouped and single individuals (group vs. single) and within a group. AIC = Akaike's information criterion; AICc = AIC corrected for small sample size.

$P = 0.09$). We found that mean TMR was twofold lower in grouped dormice compared with single individuals during hibernation (tables 6, 7, A3). However, we could not detect any effect of housing condition on mean EMR, torpid T_b , and T_{nest} (tables 6, 7, A3). Instead, T_{nest} had an effect on torpid T_b (tables 6, 7), indicating that individuals decreased their T_b during torpor close to T_a values (i.e., temperature of their nest or hibernacula; table A3).

Discussion

The present study investigated the energetic benefit of huddling behavior in hibernating juvenile garden dormice. Here, we report that being grouped is energetically advantageous during each arousal by lowering BM loss triggered by the energetically costly rewarming from torpor. Despite a clear energetic benefit during the arousal, our results of the entire hibernation period did not

show any energetic effect of huddling, as both individually and grouped hibernating animals displayed similar negative energy balance and mass loss during the winter, mainly caused by lower MR but shorter TBD in groups. Considering multiple arousals during hibernation, we noticed that animals take turn in initiating the arousal, sharing the cost of rewarming throughout the winter.

Social Thermoregulation Allows Juveniles to Save Energy during Periodic Arousals in Winter

According to BM loss during one arousal from torpor, one of our main findings demonstrates that huddling does provide substantial energetic benefits upon arousal from deep torpor in hibernating dormice, the most costly part of the torpor-arousal cycle. First, in this study we were able to estimate that arousals represent 75.8% of energy expenditure of hibernation, which is in line with other studies (Wang 1978; Willis 1982; Strijkstra 1999; Karpovich et al. 2009). Then we quantified upon-arousal BM loss and heat exchange, which are strongly related to MR, as a large proportion of metabolic heat is transferred from the body to the environment (McCafferty et al. 2011). The present study clearly demonstrates the beneficial energetic effect of social thermoregulation, or huddling, during the process of rewarming from deep torpor and provides a quantification of this benefit during a single arousal (i.e., grouped individuals lost two-thirds less BM than single individuals during rewarming; figs. 2, 4). As grouping individuals reduces the surface-to-volume ratio, heat exchange between the groups and the environment operates at a reduced rate and to a lower extent during the active process of rewarming (for review, see Gilbert et al. 2010). Consequently, energy loss from individuals within the groups is lowered. Furthermore, we found that the first individual to arouse was the only one to show a negative heat exchange (i.e., active rewarming to the environment) and hence ensured most of the energetic cost of arousal from the group, while others benefited to some extent from partial passive rewarming (illustrated by fig. 3). This finding supports Arnold (1988) who reported that heat loss in grouped alpine

Table 3: Parameters of the best models for heat exchanges and body mass change during the isolated arousal experiments

Parameter, test, variable in best model	χ^2	P
Heat exchanges:		
Group vs. single:		
Group	49.8	<.001
Within a group:		
Rank	49.1	<.001
Body mass:		
Group vs. single:		
Group	88.9	<.001
T_s end	3.8E+06	.55
Prearousal BM	3.6E-01	<.001

Note. Best models included the housing condition (group), the surface temperature at the end of the arousal (T_s end), the rank of arousal (rank), and the body mass before arousal (prearousal BM). For heat exchanges, we investigated the effect between grouped and single individuals (group vs. single) and within a group. P values shown in bold indicate statistically significant differences.

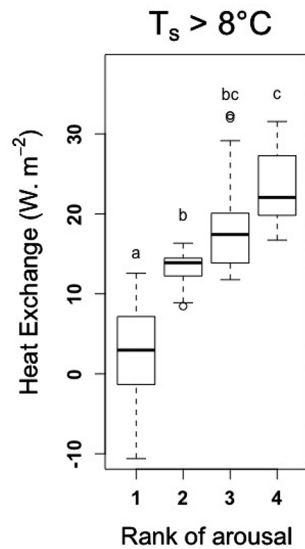


Figure 3. Boxplot of environmental heat exchange (W/m^2) for each of the four individuals within a huddle according to their rank of arousal (1–4) during the active rewarming at surface temperature (T_s) greater than 8°C , which corresponded to the temperature of the climate chamber in which the experiments took place. Different lowercase letters indicate significant ($P < 0.01$) differences between ranks. $n = 16$ individuals per rank.

marmots might be more pronounced during simultaneous euthermia and arousals. Consequently, the degree of synchronicity during the process of arousal is crucial for conferring huddling benefits, especially for reducing BM loss of hibernating individuals over winter (Ruf and Arnold 2000). It thus appears that social thermoregulation would allow animals within a huddle to save energy during both torpor and synchronized arousals but only for individuals warming passively, at the expense of the first one to arouse.

Interestingly, our investigation of the dynamics of huddling during hibernation reveals a random-like mechanistic behavior occurring in winter, as group arousals from torpor were not always initiated by the same individuals. Surprisingly, the mean T_b during interbout euthermia and the order of cooling from the previous arousal, although nonsignificant, were included in the first models explaining the rank of arousal from torpor. Furthermore, it seems that individuals with a high T_b during euthermic intervals entered into torpor later among the individuals within the huddle and warmed later as well at the following arousal, hence benefiting from passive rewarming. Hence, these variables might play a role in determining the dynamics of rewarming and cooling. However, these models altogether explained just 30% of the variance, which highlights the possibility that other unmeasured parameters, such as social interactions, may explain the rank of arousal or cooling. Indeed, the dynamics inside a group could be an indirect way to improve the fitness of relatives, as recently suggested by Ruf and Bieber (2020), for hibernating edible dormice (*Glis glis*). In the present study, groups were randomly composed and animals were grouped with unrelated

individuals, which differs from the natural situation. Hence, in future studies, it might be interesting to include brotherhood or litter origin for each individual and to determine the specific effects of such grouping on the dynamics of huddling during hibernation. In the case of animal groups with mixed ages, the biggest individuals (i.e., parents) may be more likely to arouse first, hence providing energetic benefits to the youngest individuals in the nest (Arnold 1988). Also, the position of the animals within the huddle or in the litter might play a role in the dynamics of rewarming. Indeed, differences in brown fat metabolism have been reported to occur in newborn rats according to their position in the litter, with less competitive individuals occupying the peripheral positions (Schank and Alberts 1997) showing a higher brown fat metabolism. Furthermore, Bautista et al. (2013) reported that peripheral rabbit pups show greater expression of UCP-1, a mitochondrial protein involved in nonshivering thermogenesis, lowering mitochondrial efficiency (ATP synthesis) and reducing energy saving. Hence, it might be relevant to investigate the dynamics of rewarming according to the position in the litter with bigger groups and possible long-term effects of differences in brown fat metabolism induced during postnatal days. In light of the current literature, our investigation of the dynamics of huddling during hibernation strongly suggests the existence of a complex behavior of social grouping and interactions in hibernating species during winter.

Group Dynamics Seems to Counterbalance Energetic Benefits of Social Thermoregulation during Winter

In this study we found no energetic benefit of huddling during the entire winter hibernation, as we did not detect any difference

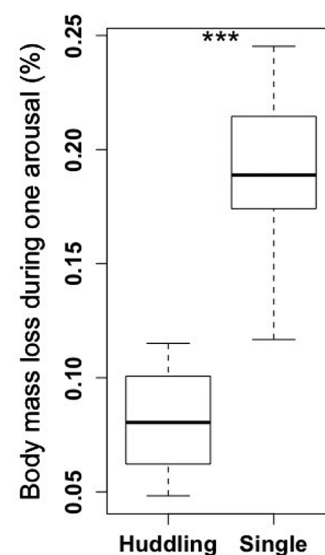


Figure 4. Boxplots of body mass loss calculated in percentage of pre-arousal body mass from dormice housed singly (single) or in groups of four individuals (huddling) during one standardized rewarming. $n = 27$ individuals: 11 single and 16 grouped individuals. *** $P < 0.001$.

Table 4: Best candidate models ($\Delta AICc < 2$) explaining the proportion to rewarm first, the rank of arousal, and the rank of cooling over winter during hibernation in garden dormice

Parameter, model	AICc	ΔAIC	Weight
Proportion to rewarm first:			
Hib duration	-14.1	.00	.18
Mean T_b	-13.2	.91	.11
Total EBD	-12.5	1.59	.08
Rank of arousal:			
Hib duration	591.9	.00	.06
Rank cooling + hib duration	591.6	.49	.05
Mean T_b + hib duration	592.0	.88	.04
Prehibernation BM + hib duration	593.0	1.87	.02
EBD + hib duration	593.1	1.99	.02
Mean T_b + rank cooling + hib duration	593.1	1.99	.02
Rank of cooling:			
Mean T_b + hib duration	650.4	.00	.10
Mean T_b + TBD + hib duration	651.7	1.25	.05
Mean T_b + EBD + hib duration	651.9	1.49	.05
Mean T_b + hib duration + prehibernation BM	652.1	1.62	.05
Mean T_b + hib duration + sex	652.4	1.98	.04

Note. Explanatory variables were total hibernation length (hib duration), mean body temperature during single interbout euthermia (mean T_b), body mass before hibernation (prehibernation BM), sex, single euthermia duration (EBD), total EBD, torpor bout duration (TBD), and rank of cooling. AIC = Akaike's information criterion; AICc = AIC corrected for small sample size.

in BM loss between housing conditions. However, we did not determine body composition, such as fat content, depending on housing condition, which might be a complementary aspect to investigate (see below for details). Moreover, we investigated only the first winter of our animals and some long-term effects can also occur (i.e., after several winters) along the individual's life. Also, we could not find a major effect of huddling on torpor use during winter. Although grouped individuals reduced total torpor duration compared with single dormice during winter, no difference between grouped and solitary individuals was detected for mean TBD or arousal frequency. A reduced torpor utilization by groups has already been demonstrated in striped skunks (*Mephitis mephitis*), where solitary individuals entered torpor on average

10 times more than grouped ones and spend 1.5 times longer in torpor (Hwang et al. 2007). Such an effect can be explained by interactions between individuals within a huddle (Dausmann and Glos 2014), notably through a longer cooling process. Indeed, the temperature of the air surrounding an individual is to a large extent a function of the size of the group, so the cooling process during entry into torpor is slower within a group than for isolated individuals. Moreover, some individuals within a group might show partial rewarming during entry into torpor, which may disturb the cooling process as well (Ruf and Arnold 2000; this study). A reduced torpor use by huddling animals might also provide other benefits, such as fewer physiological damages triggered by oxidative stress (Von Zglinicki 2002) or lower sleep disturbance (Strijkstra and Daan 1997; Royo et al. 2019).

Although no effect on BM of hibernating in a group was found during winter, our results revealed that grouped individuals showed a lower TMR compared with single individuals, indicating an enhanced energy saving during torpor through huddling. Similar observations were reported in other species, such as the white-footed mouse (*Peromyscus leucopus*), where huddling reduces MR of grouped mice exposed to different T_a even more if used in synergy with torpor (Vogt and Lynch 1982; Vogt and Kakooza 1993). Grouped individuals showed an increase in energy saving by 11% compared with isolated ones and by 17% when torpid, and the extent of energy saving was dependent on T_a , with greater energetic benefits provided by grouping at higher T_a (Vogt and Lynch 1982; Vogt and Kakooza 1993). In our study, however, T_a remained low and constant during the entire winter, and it is well known that hibernating at increased T_a reduces energy saving during hibernation (French 1982; Geiser

Table 5: Parameters of the best model for the dynamics inside a huddle

Parameter, variable	χ^2	P
Proportion to rewarm first:		
Hib duration	.01	.98
Rank of arousal:		
Hib duration	.01	.94
Rank of cooling:		
Hib duration	.15	.70
Mean T_b	7.30	.006

Note. We studied the effect of total hibernation length (hib duration) and mean body temperature during interbout euthermia (mean T_b). The P value shown in bold indicates statistically significant differences.

Table 6: Best models ($\Delta\text{AICc} < 2$) for variables of hibernating patterns

Parameter, model	AICc	ΔAIC	Weight
Total TBD:			
Group + hib duration	645.29	.00	.78
Total EBD:			
Group + sex + hib duration	240.11	.00	.38
Group + hib duration	240.55	.44	.31
Arousal frequency:			
Hib duration	129.82	.00	.45
Sex + hib duration	130.35	.52	.35
Min T_b :			
T_{nest}	-16.19	.00	.42
Mean TMR:			
Group	-102.79	.00	.71

Note. Dependent variables included total torpor bout duration (total TBD), total interbout euthermia duration (total EBD), arousal frequency, minimum body temperature during torpor (min T_b), and mean torpid metabolic rate (mean TMR). We investigated the effect of total hibernation length (hib duration), sex, temperature of the nest (T_{nest}), and housing condition (group). AIC = Akaike's information criterion; AICc = AIC corrected for small sample size.

and Kenagy 1988; Bieber and Ruf 2009; Nowack et al. 2019). Indeed, MR during torpor is maintained according to the $T_b - T_a$ differential (for review, see Geiser 2004), as demonstrated in a marsupial hibernator, the mountain pygmy possum (*Burramys parvus*), initially hibernating at 2°C and showing increased TMR when T_a increases during winter (Geiser and Broome 1993). However, when T_a decreases to 0°C and below, TMR increases to maintain T_b at a minimal physiological value, as reported in mountain pygmy possums (Geiser and Broome 1993) and arctic ground squirrels (*Spermophilus parryii*; Buck and Barnes 2000). In our study no difference in core T_b during torpor was found between housing conditions despite a lower TMR in groups. As individuals remained in a close aggregation in a posture that reduces heat loss during torpor, a reduced MR might not be associated with a lower T_b and therefore may not help to avoid physiological damages. It thus appears that being grouped would be energetically advantageous when T_a reaches low temperatures (near 0°C or below) by keeping the microenvironment warmer and T_b above the minimum physiological value (Geiser and Broome 1993; Buck and Barnes 2000; Gilbert et al. 2010).

The lower MR of grouped individuals during winter might be related to modulations in gut microbiota, as suggested by Zhang et al. (2018) in Brandt's voles. More precisely, social thermoregulation is proposed to increase the diversity of cecal microbiota. It triggers changes in the fermentation of metabolites, such as acetic, butyric, and propionic acids, which are known to regulate metabolic pathways, including energy intake, appetite, and thermogenesis (Li et al. 2018). It has also been suggested that social behavior affects the taxonomic and genic composition of animal microbiomes. Indeed, a study made on Brandt's voles (*Lasiopodomys brandtii*) reported that huddling (along with cold) resulted in modifications of microbiota abundance compared

with separated voles and was followed by higher concentrations of short-chain fatty acids (Zhang et al. 2018). Consequently, the specific changes of the gut microbiota, orchestrating metabolic and thermal homeostasis, may constitute a likely explanation for the lower MR in torpor of huddled juveniles compared with single individuals in our study. Such changes in metabolic pathways might also be associated with shifts in metabolic substrates (Li et al. 2018) and hence changes in body composition. In our hibernating dormice, body composition could have been affected differently in hibernating individuals in groups versus single individuals, with higher use of lipid substrate and hence greater sparing of proteins (i.e., muscle mass) during hibernation (for review, see Bertile et al. 2021). Such an aspect would be of interest for future investigations, in particular given the importance of the maintenance of muscle mass in heterothermic species for subsequent reproductive success of individuals (Giroud et al. 2010; Mahler et al. 2018).

Finally, active rewarming implies increases of MR and T_b , resulting in massive \dot{V}_{O_2} triggering high risk of oxidative stress, as oxygen reactive species are overproduced when mitochondrial respiration is uncoupled, which happens during rewarming via nonshivering thermogenesis (Carey et al. 2003). A study in bats shows a reduced \dot{V}_{O_2} during passive compared with active rewarming; it suggests that grouped individuals reducing active arousal time (passive rewarming and higher T_b) may minimize their exposure to oxidative stress (Currie et al. 2015). Consequently, huddling during hibernation and social rewarming may be a way to reduce cellular and molecular damages related to increase in oxidative stress. Taking turns in initiating rewarming would allow individuals to successively rewarm passively and to share that cost throughout winter. Also, by maintaining a warmer

Table 7: Parameters of the best models for variables of hibernating patterns

Parameter, variable	χ^2	<i>P</i>
Total TBD:		
Group	11.56	<.001
Hib duration	34.05	<.001
Total EBD:		
Group	7.01	.06
Sex	1.28	.13
Hib duration	3.72	.07
Arousal frequency:		
Hib duration	8.79	.003
Min T_b :		
T_{nest}	465.37	<.001
Mean TMR:		
Group	15.74	<.001

Note. Parameters were total torpor bout duration (total TBD), total interbout euthermia duration (total EBD), arousal frequency, minimum body temperature (min T_b) during torpor, and mean torpid metabolic rate (mean TMR). We investigated the effect of total hibernation length (hib duration), sex, temperature of the nest (T_{nest}), and housing condition (group) on the different dependent variables of hibernating patterns. *P* values shown in bold indicate statistically significant differences.

microenvironment and lower MR during winter, being grouped might reduce overall mitochondrial metabolism and reactive oxygen species production (Brown et al. 2012; Currie et al. 2015). It appears that physiological and oxidative status of individuals should be considered to better understand the dynamics of social thermoregulation.

According to our investigations, it seems that garden dormice take turns in initiating rewarming within a group during winter, therefore sharing both costs and benefits of huddling during periodic arousals. So why do the animals in the wild huddle during winter hibernation? As suggested above, hibernating in groups can be seen as a way to decrease risk of predation via a dilution effect (Gilbert et al. 2010), to facilitate social harmony, to structure social interactions between individuals, and, in nature, to enhance prospects for mating and breeding (Kunkel 1974; Wagner et al. 2000), hence providing long-term energetic benefit. Taken together, our observations suggest that social thermoregulation during winter in heterothermic species is not only driven by energetic constraints but also might be strongly related to social interactions, offspring survival, physiological damages, and sleep debt.

Conclusion

This study emphasizes the plasticity of energy-saving strategies, such as torpor and huddling, used by the garden dormouse. Because of current global changes and the increasing occurrence of unpredictable events, it is highly relevant to determine the extent to which the flexibility of energy-saving strategies will enable individuals to survive seasonal winter hibernation. In the present study, our hypothesis about huddling providing energy savings was partially fulfilled. Huddling did reduce energy expended during rewarming, the most energy-costly phase of the torpor-arousal cycle. However, the use of huddling did not buffer BM loss in juveniles over the entire hibernation. Interestingly, animals took turns in rewarming during winter, which might counterbalance the advantages of huddling. As a follow-up to our study, it would thus be interesting to modify group composition to focus on huddling strategy, such as nesting of relatives with both juveniles and adults, and study possible long-term effects after several winters. Other benefits such as physiological damages or sleep debt would also deserve further investigations. Advancing on all of these hypotheses would allow investigation of whether there are some energetic benefits of social thermoregulation under natural conditions during winter hibernation and what the causes for and consequences of group formation are in this species.

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The raw data of this study will be made available by us to any qualified researcher upon request and without undue reservation. S.G. and C.G. conceived and designed the study. J.E. and J.P. performed surgeries for implantation of temperature loggers. L.M.C. and S.G. performed the experiments, and L.M.C. computed the data under the supervision of S.G. L.M.C. and S.V. performed statistical analyses. L.M.C. drafted the manuscript. S.G. and C.G. substantially edited and critically revised the manuscript. All coauthors commented on the manuscript and agreed on its content. S.G. was financially supported by the Austrian Science Fund (grant P31577-B25) and the Austrian Agency for International Cooperation in Education and Research (Scientific and Technological Cooperation grant FR 09/2020). We declare that the presented research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX

Surgical Implantations of Loggers and Temperature Measurements

Surgical anesthesia was induced by a subcutaneous injection of 50 mg/kg ketamine (Ketamidol ND 10%, Richter Pharma, Wels, Austria) and 8 mg/kg xylazine (Rompun ND 2%, Bayer, Leverkusen, Germany). Anesthesia was maintained on a small-animal surgery table unit (combi-vet surgery table, Rothacher Medical, Heitenried, Switzerland) by using 1.5% isoflurane (Vetflurane, Virbac, Vienna, Austria) gas in an oxygen stream via a specially designed face mask. Temperature was controlled by an integrated safe heating plate (combi-vet) including a gas evacuation system. Postoperative analgesia was achieved by using 2 mg/kg meloxicam (Metacam, Boehringer Ingelheim, Schweiz) presurgically in a subcutaneous injection. The operation field was prepared according to standard surgical procedures and covered by sterile surgical drapes. Animals were placed in dorsal recumbency, and the abdominal cavity was opened through a 1-cm incision in the linea alba to introduce the temperature logger within the abdomen. The abdominal fascia was sutured using synthetic absorbable surgical suture material USP 3-0 (Surgicryl PGA, SMI AG, Hünningen, Belgium) using a single-button suture technique. In addition, synthetic absorbable surgical suture material USP 4-0 (Surgicryl PGA) was used to suture the submucosal and skin layer with a continuous noninterrupted intracutaneous suture technique. During the entire procedure, vital parameters (body temperature, respiration rate, peripheral hemoglobin oxygen saturation as measured by pulse oximetry [SpO₂], heart rate) were monitored.

Respirometry for Measurements of Metabolic Rate

Flow rates of approximately 40 L/h for single individuals and 80 L/h for groups were used and continuously measured using calibrated thermal mass flow meters (FMA 3100, Omega Engineering, Stamford, CT). Oxygen concentration was determined by a dual-channel electrochemical O₂ analyzer (Oxzilla FC-2

differential oxygen analyzer, Sable Systems, Las Vegas, NV), which was calibrated once before the experiments with nitrogen for zero-oxygen values. During the hibernation experiments, we used the autocalibration function of Oxzilla to reset the oxygen concentration to reference air at regular intervals. Air was pumped through the respiratory chamber (pulled mode) with membrane pumps. All recordings were interfaced to a laptop. Relative humidity was measured in sampled air and used for correction within the calculations. O₂ data were corrected for drift of the analyzer by

automated switching to sample reference air at regular intervals. MR (i.e., \dot{V}_{O_2} ; L/h) was calculated using the following equation:

$$\dot{V}_{O_2} = FD \times \frac{FIO_2 - FEO_2}{(1 - FIO_2) \times (1 - 0.70)},$$

where FD is dry flow, FIO₂ is fractional concentration of O₂ in the incoming airflow, and FEO₂ is fractional concentration of O₂ in the outgoing airflow, assuming a respiratory quotient of 0.7 (Lighton 2008).

Table A1: Physiological parameters and treatment of animals included in the experiments

ID	Sex	Year	Time of birth	Food treatment	Housing condition	BM prehibernation (g)	BM posthibernation or postarousal (g)	Experiment(s)
9409	F	2018–2019	LB	AL	Huddling	77.5	63	DYN
9832	F	2018–2019	LB	AL	Huddling	84.5	67.5	DYN
9953	F	2018–2019	LB	AL	Huddling	91.5	72.5	DYN
9959	F	2018–2019	LB	AL	Huddling	92	72.5	DYN
9418	F	2018–2019	LB	Fast	Huddling	85.5	66	HIB, DYN, MR
9834	F	2018–2019	LB	Fast	Huddling	83.5	69.5	HIB, DYN, MR
9952	F	2018–2019	LB	Fast	Huddling	73	58	HIB, DYN, MR
9958	F	2018–2019	LB	Fast	Huddling	81	65	HIB, DYN, MR
9417	F	2018–2019	LB	AL	Huddling	79	65	DYN
9420	F	2018–2019	LB	AL	Huddling	74	56	DYN
9908	F	2018–2019	LB	AL	Huddling	76	59	DYN
9915	F	2018–2019	LB	AL	Huddling	76.5	62	DYN
9830	F	2018–2019	LB	Fast	Huddling	82.5	61	HIB, DYN
9948	F	2018–2019	LB	Fast	Huddling	74.5	55	HIB, DYN
9954	F	2018–2019	LB	Fast	Huddling	90.5	68	HIB, DYN
9964	F	2018–2019	LB	Fast	Huddling	76	57.5	HIB, DYN
9831	M	2018–2019	LB	Fast	Huddling	93	73	HIB, DYN, MR
9839	M	2018–2019	LB	Fast	Huddling	81	62.5	HIB, DYN, MR
9957	M	2018–2019	LB	Fast	Huddling	92.5	70	HIB, DYN, MR
9965	M	2018–2019	LB	Fast	Huddling	85.5	66	HIB, DYN, MR
9407	M	2018–2019	LB	Fast	Huddling	86	62.5	HIB
9837	M	2018–2019	LB	Fast	Huddling	90	69.5	HIB
9955	M	2018–2019	LB	Fast	Huddling	93	62	HIB
9963	M	2018–2019	LB	Fast	Huddling	85.5	58	HIB
9419	F	2018–2019	LB	Fast	Single	72.5	56.5	HIB, MR
9829	F	2018–2019	LB	Fast	Single	75.5	58.5	HIB, MR
9909	M	2018–2019	LB	Fast	Single	114	83.5	HIB, MR
9902	M	2018–2019	LB	Fast	Single	83	60.0	HIB, MR
3961	F	2019–2020	LB	Fast	Single	80.5	62.0	HIB
3963	F	2019–2020	LB	Fast	Single	92.5	69.6	HIB
4015	F	2019–2020	LB	Fast	Single	65.0	48.7	HIB
4063	F	2019–2020	LB	Fast	Single	79.5	56.4	HIB
3966	M	2019–2020	LB	Fast	Single	96.5	74.3	HIB
4017	M	2019–2020	LB	Fast	Single	98.5	73.3	HIB
4069	M	2019–2020	LB	Fast	Single	79.5	61.1	HIB
4187	M	2019–2020	LB	Fast	Single	110.5	86.1	HIB
4067	M	2019–2020	LB	Fast	Single	96.5	75.4	HIB
4063	F	2019–2020	LB	Fast	Single	79.5	56.4	HIB

Table A1 (Continued)

ID	Sex	Year	Time of birth	Food treatment	Housing condition	BM prehibernation (g)	BM posthibernation or postarousal (g)	Experiment(s)
3967	F	2019–2020	LB	Fast	Single	75.0	58.8	HIB
3935	M	2019–2020	EB	...	Huddling	124.5	102.4	AR
3977	M	2019–2020	EB	...	Huddling	133.5	78.6	AR
3996	M	2019–2020	EB	...	Huddling	122.0	99.2	AR
4119	M	2019–2020	EB	...	Huddling	123.5	95.5	AR
3924	M	2019–2020	EB	...	Huddling	101.5	82.8	AR
3997	M	2019–2020	EB	...	Huddling	98.5	78.6	AR
4083	M	2019–2020	EB	...	Huddling	98.5	78.8	AR
4088	M	2019–2020	EB	...	Huddling	110.0	85.8	AR
4085	M	2019–2020	EB	...	Single	113.0	89.9	AR
4087	M	2019–2020	EB	...	Single	118.0	93.2	AR
4198	M	2019–2020	EB	...	Single	108.0	89.5	AR
3995	M	2019–2020	EB	...	Single	102.5	78.6	AR
3941	M	2019–2020	EB	...	Single	132.5	102.8	AR
3937	M	2019–2020	EB	...	Huddling	117.0	92.3	AR
3943	M	2019–2020	EB	...	Huddling	114.0	90.2	AR
3992	M	2019–2020	EB	...	Huddling	112.0	88.4	AR
4082	M	2019–2020	EB	...	Huddling	110.0	89.4	AR
3989	M	2019–2020	EB	...	Huddling	122.5	103.3	AR
3999	M	2019–2020	EB	...	Huddling	108.5	93.2	AR
4200	M	2019–2020	EB	...	Huddling	97.5	79.5	AR
4212	M	2019–2020	EB	...	Huddling	116.5	94.3	AR
3951	M	2019–2020	EB	...	Single	115.0	92.6	AR
4081	M	2019–2020	EB	...	Single	102.0	80.1	AR
4115	M	2019–2020	EB	...	Single	108.5	90.2	AR
4000	M	2019–2020	EB	...	Single	116.5	93.0	AR
3980	M	2019–2020	EB	...	Single	155.0	119.8	AR
3994	M	2019–2020	EB	...	Single	132.5	102.8	AR

Note. Animals from 2018–2019 and 2019–2020 were included in the experiments of hibernation pattern and body mass loss (HIB), dynamics inside a group (DYN), metabolic rate (MR), and isolated arousal (AR). Animals were either early born (EB) or late born (LB) in the reproductive season. For all experiments, we tested for the effect of sex (F for female, M for male) and housing condition (either single or huddling in groups of four individuals, unisex). For DYN, we studied the effect of food treatment, as individuals were hibernating without food (fast) or with food ad lib. (AL). For HIB, all individuals were fasted. Animals were weighed at prehibernation and after winter hibernation for LB juveniles or after arousal for EB individuals. Individual ID was included as a random effect to account for repeated measurements among animals. $n = 66$.

Table A2: Comparison of body masses between single or grouped male and female juvenile garden dormice during the whole-winter experiments (October 2018 and 2019)

	Single	Grouped	Statistics	
			Single vs. grouped	Males vs. females
Prehibernation body mass (g)	$t = -3.8, P < .01$
Males ($n = 15$)	91.0 ± 10.1	90.0 ± 9.6	$t = -.1, P = .75$...
Females ($n = 16$)	78.8 ± 7.9	82.8 ± 7.8	$t = 1.6, P = .12$...
Posthibernation body mass (g)	$t = -4.2, P < .01$
Males ($n = 15$)	71.7 ± 2.6	69.4 ± 2.1	$t = -.7, P = .50$...
Females ($n = 16$)	59.7 ± 2.4	62.5 ± 1.9	$t = .9, P = .40$...

Note. P values shown in bold indicate statistically significant differences. Values are mean \pm SE.

Table A3: Parameters of hibernating patterns

Parameter, test	Mean \pm SE	<i>P</i>
Total TBD (h)	...	<.001
Single (15)	2,530.7 \pm 39.1	...
Grouped (16)	2,449.7 \pm 25.1	...
Total EBD (h):		
Single36
Males (7)	67.3 \pm 13.7	...
Females (8)	68.9 \pm 11.6	...
Grouped48
Males (8)	71.8 \pm 10.5	...
Females (8)	90.7 \pm 10.9	...
Arousal frequency11
Males (15)	10.7 \pm .9	...
Females (16)	12.2 \pm .9	...
Torpid T_b ($^{\circ}\text{C}$)	...	<.001
T_{nest}	3.3 \pm .1	...
T_{nest} ($^{\circ}\text{C}$)19
Single (15)	3.4 \pm .5	...
Grouped (16)	3.1 \pm .6	...
Mean TMR (mL/h)004
Single (15)	.04 \pm .005	...
Grouped (6)	.02 \pm .0008	...

Note. Variables of the best model included total torpor bout duration (TBD), total interbout euthermia duration (EBD), arousal frequency, minimum body temperature during torpor (torpid T_b), and mean torpid metabolic rate (TMR). We investigated the effect of total hibernation length, sex, temperature of the nest (T_{nest}), and housing condition (grouped). Sample sizes are indicated in parentheses. *P* values shown in bold indicate statistically significant differences.

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