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Torpor initiation and metabolic regulation operate at different biological levels in coexisting temperate bats

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Abstract

Understanding how organisms balance energy use across biological levels is central to explaining physiological responses to shared environmental constraints. In small endotherms, torpor represents a key energy-saving strategy, yet how coexisting species differ in torpor expression remains poorly understood. Here, we evaluated whether torpor use, torpor depth, and normothermic metabolic rate were associated with between- and within-species variation in body mass (M_b) in coexisting insectivorous bats from a temperate ecosystem in central Mexico. Using phylogenetic and non-phylogenetic mixed models, we partitioned M_b into between- and within-species components and assessed the effects of energetic state (energy-supplied vs. energy-restricted) and ambient temperature (T_a) under controlled conditions. Torpor use was explained primarily by interspecific variation in M_b, with larger species showing a higher probability of entering torpor, whereas phylogeny explained negligible variation. In contrast, both torpor depth and normothermic metabolic rate were driven by intraspecific variation in M_b, with smaller individuals achieving lower torpid metabolic rates. Torpor depth was further modulated by an interaction between energetic state and T_a, indicating that the magnitude of metabolic suppression depends on both environmental conditions and individual energetic status. Together, these results reveal a decoupling between the determinants of torpor use and metabolic regulation operating through distinct between- and within-species processes. These findings suggest that variation in torpor expression represents an important axis of physiological differentiation among coexisting species and provide a framework for investigating how physiological strategies contribute to ecological coexistence under fluctuating environmental conditions.

Introduction

Organisms continuously experience environmental variation that influences the balance between energy acquisition and expenditure (Becker et al. 2013). In small endotherms, these challenges are particularly pronounced because maintaining homeothermy is energetically expensive due to their high mass-specific metabolic rates (Körtner and Geiser 2000; Geiser et al. 2018). When environmental conditions reduce energy availability or increase thermoregulatory costs, many species enter torpor, a reversible state of reduced metabolic rate and body temperature that substantially decreases energetic expenditure (Geiser and Ruf 1995; Geiser 2004; Heldmaier et al. 2004; Dikic et al. 2008; Geiser 2010). Beyond the decision to enter torpor, individuals may also differ in torpor depth, reflected by the magnitude of metabolic suppression achieved during torpor (Speakman and Thomas 2003; Ayala-Berdon and Medina-Bello 2025). Because torpor depth determines the extent of energy savings, variation in this trait represents an additional axis of physiological flexibility. Consequently, torpor is one of the most effective physiological strategies for coping with energetic challenges, with important consequences for survival, reproduction, and species distributions.

Previous comparative studies have shown that torpor use is influenced by multiple intrinsic and extrinsic factors (Racey and Swift 1981; Lovegrove et al. 1999; Wilde et al. 1999; Chruszcz and Barclay 2002;

Geiser and Turbill 2009; Körtner and Geiser 2009; Pretzlaff et al. 2010). Body mass (M_b) influences both metabolic demands and the energetic costs associated with thermoregulation, whereas the energetic state of individuals affects the probability of entering torpor. Ambient temperature (T_a) modifies both torpor initiation and the extent of metabolic suppression (Brown et al. 2004). Together, these studies demonstrate that torpor expression varies considerably both among bat species and among individuals within species. However, few studies have explicitly distinguished the effects of M_b operating among species from those operating among individuals within species.

Distinguishing these sources of variation is important because M_b varies both among and within species, potentially influencing energetic responses through different physiological mechanisms. Evaluating interspecific and intraspecific responses separately prevents direct assessment of the relative contribution of these biological levels to variation in torpor expression. Because M_b reflects both evolutionary differences among species and phenotypic variation among individuals, investigating these components may reveal whether torpor use is primarily associated with species-specific strategies or individual physiological condition.

This distinction is particularly relevant in bats, a group that exhibits remarkable diversity in torpor regulation despite sharing many physiological characteristics. Comparative studies have shown that torpor varies among bat species according to body size, environmental conditions, and energetic state, whereas experimental studies within species have emphasized the roles of food availability and T_a in modulating torpor expression (Vuarin and Henry 2014). Insectivorous bats provide an ideal system for disentangling interspecific and intraspecific determinants of torpor among coexisting species. As small-bodied endotherms with high mass-specific metabolic rates, bats experience substantial energetic demands and rely heavily on flexible physiological strategies, including torpor (Ransome 1990; Kunz et al. 1995; Chruszcz and Barclay 2002; Ayala-Berdon and Medina-Bello 2024). Many temperate insectivorous bats use torpor regularly, although species differ markedly in M_b, roosting ecology, and foraging behavior (Speakman and Thomas 2003; Ayala-Berdon and Medina-Bello 2024). Evaluating these responses under standardized laboratory conditions allows physiological differences to be evaluated independently of short-term environmental variation. Therefore, distinguishing the relative contributions of interspecific and intraspecific variation in M_b provides an opportunity to identify the biological level at which the determinants of torpor expression primarily operate.

Here, we investigated whether the mechanisms underlying torpor expression differ between species and individuals within species in a community of insectivorous bats from a temperate ecosystem in central Mexico. We hypothesized that the determinants of torpor use, torpor depth, and normothermic metabolic rate operate at different biological levels. To test this hypothesis, we partitioned M_b into between-species and within-species components and evaluated their independent effects while accounting for energetic state, T_a, and phylogenetic relationships.

Methods

Study site

All bats were captured during the dry-cold season (October 2019- February 2020), in Santa Cruz Moxolahuac, a montane ecosystem located in Puebla, central Mexico (3,121 m a.s.l.; 19° 27' 2.188" N, 98° 34' 10.131" W) (Fig. 1), thereby minimizing seasonal variation among species. The area is characterized by a cold temperate subhumid climate, with vegetation dominated by coniferous forest, including oyamel (*Abies religiosa*), Hartweg's pine (*Pinus hartwegii*), and Moctezuma pine (*Pinus montezumae*). These species structure the canopy and generate particular microclimatic conditions, including low ambient temperatures (T_a) and high humidity. During the study, mean T_a and precipitation were 14.0 ± 4.7°C, and 1,800 ± 15 mm, respectively (mean ± SE).

Experimental design

Bats were captured using mist nets (2 × 6 and 2 × 12 m) placed over a water body used for foraging and drinking. Nets were opened at dusk and closed around 24:00 h. A total of 21 adult male individuals (non-reproductive) distributed in six species were selected, as follows: *Myotis volans* (n = 6), *Lasiurus cinereus* (n = 4), *Eptesicus fuscus* (n = 4), *Lasiurus ega* (n = 3), *Myotis thysanodes* (n = 2), and *Myotis californicus* (n = 2). For each individual, body mass (M_b) and forearm length were recorded using a digital scale (Ohaus, Newark, New Jersey, USA; ± 0.2 g) and a caliper (± 0.1 mm), respectively. Body mass was measured immediately after capture and before each experimental trial. The value recorded prior to each trial was used in the corresponding statistical analyses. Age was estimated by examining the epiphyseal gap in the fourth metacarpal of the third and fifth digits (Wilkinson and Brunet-Rossinni 2009). After capture, individuals were transported in cloth bags to experimental facilities and housed in flight cages (75 × 75 × 75 cm) under controlled conditions (12 h:12 h light–dark cycle, ambient temperature ~ 28°C, relative humidity > 50%). Water was provided *ad libitum* to prevent dehydration.

The experimental protocol followed a standardized sequence (Fig. 2). Following capture, bats were maintained under controlled laboratory conditions and were subjected to a 6–8 h fasting period to ensure a post-absorptive state. On the following day, basal metabolic rate (*BMR*, as the minimum metabolic rate required to sustain basic biological functions at rest), and the lower (T_{LC}) and upper (T_{UC}) critical temperatures delimiting the thermoneutral zone were measured under standardized conditions (Genoud et al. 2018).

After completion of the euthermic measurements, torpor expression was evaluated using a repeated-measures design under two energetic treatments. In the energy-supplied treatment, individuals were fed mealworms (*Tenebrio molitor*) *ad libitum* at approximately 20:00 h, corresponding to the natural foraging period of insectivorous bats (Rydell et al. 1996; Grindal and Brigham 1999). Torpor measurements were performed during the subsequent inactive phase, approximately 12–13 h after feeding. Following the completion of the energy-supplied trial, the same individuals underwent energy-restricted treatment consisting of a 36 h food deprivation period, simulating short-term energetic limitation under natural

conditions (Grindal and Brigham 1998). This interval included the intermediate inactive phase so that torpor was assessed at the same circadian stage as in the energy-supplied treatment. Torpor was defined based on metabolic criteria (see definition below), as body temperature was not measured directly; sustained reductions in metabolic rate are widely accepted as a proxy for torpor expression in bats.

Each individual was measured under both energetic treatments and therefore served as its own control, minimizing their inter-individual variability and increasing statistical power to detect treatment effects. The order of treatments was not randomized; all bats completed the energy-supplied trial before the energy-restricted trial to avoid prolonged fasting immediately after capture and to standardize the duration of food deprivation across individuals. Because all species were sampled from the same seasonal period, comparisons were made under comparable environmental conditions, allowing physiological differences among species and individuals to be evaluated independently of short-term environmental variation. All procedures were conducted under permits issued by the Ministry of Environment and Natural Resources of Mexico (SEMARNAT: SGPA/DGVS/06795/21) and were approved by the ethics committee of the Autonomous University of Tlaxcala.

Respirometry

Metabolic measurements were conducted using open-flow indirect calorimetry (FoxBox®, Sable Systems International, Las Vegas, NV, USA), simultaneously quantifying O_2 consumption and CO_2 production, allowing accurate estimation of metabolic rate in terrestrial endotherms (Lighton 2018). Each bat was placed in a 410 mL metabolic chamber located inside a temperature-controlled cabinet (Pelt-drop-in®, Sable Systems International). To prevent flight activity while maintaining a natural posture, a plastic mesh was placed inside the chamber. Ambient temperature was maintained within ± 0.5°C using a high-precision controller.

CO_2-free air, previously scrubbed using Drierite (calcium sulfate) and Ascarite (sodium hydroxide), was pushed at a flow rate of 150–350 mL min^{-1}, adjusted according to species-specific metabolic rates (Lighton and Halsey 2011). Flow rates were verified using a calibrated flowmeter. Excurrent air was dried prior to analysis, and fractional concentrations of O_2 (FeO_2) and CO_2 ($FeCO_2$) were recorded continuously at 1 s intervals. To correct for instrumental drift, an empty chamber of equivalent volume (410 mL) was run in parallel under the same conditions using a second calibrated FoxBox® system. Data from this reference chamber were used to adjust baseline measurements.

Euthermic traits

Euthermic measurements were conducted prior to torpor trials to determine basal metabolic rate (*BMR*) and the limits of the thermoneutral zone (lower critical temperature, T_{LC}; and upper critical temperature T_{UC}). Each bat was initially maintained at 28°C (within the thermoneutral zone) for 60 min to allow

stabilization, followed by a 30 min recording period to estimate BMR (Ayala-Berdon and Medina-Bello 2024). To determine T_{LC}, T_a was gradually reduced from 28 ºC to 8°C. Metabolic rate was recorded for 30 min at each 5°C decrement and for 5 min at each 1°C decrement. If an individual entered torpor during cooling, T_a was immediately returned to 28 ºC and the protocol was restarted to ensure that all measurements represented euthermic metabolism. Following completion of the cooling trial, T_a was maintained at 28°C for 30 min before being gradually increased to 43°C using the same recording schedule to estimate T_{UC}. Throughout these measurements, all individuals remained normothermic, as indicated by the expected increase in metabolic rate below T_{LC} and above T_{UC}. These measurements provided the physiological reference values subsequently used to identify torpor during the experimental trials (Genoud et al. 2018; Speakman and Thomas 2003; Geiser 2021).

Torpor trials

Torpor use was evaluated under the two energetic treatments described above (Fig. 2). For each trial, individuals were placed in the metabolic chamber at 28°C for 60 min to allow stabilization, followed by a 75 min recording period under thermoneutral conditions. Ambient temperature was then gradually reduced from 28 to 8°C. During cooling, metabolic rate was recorded continuously, with measurements every 1°C (5 min) and extended recording periods of 75 min at 23, 18, 13, and 8°C. After final recording at 8°C, T_a was returned to 28°C and maintained for 30 min to facilitate rewarming before the individual was removed from the chamber. The same protocol was repeated under both energetic treatments for each individual. Individuals were maintained under laboratory conditions only for the duration required to complete the experimental protocol, minimizing potential acclimation effects. No mortality occurred during experiments, and all individuals were released at their capture site after completion of the trials.

Metabolic calculations

All analyses were conducted in R (version 4.5.2) with RStudio (Version 2026.01.1 + 403). Metabolic rate was calculated using O_2 consumption ($\dot{V}O_2$) (in mL O_2 h^{-1}) (Lighton 2018):

$$\dot{V}O_2 = FR\ [(FiO_2 - FeO_2) - FeO_2(FeCO_2 - FiCO_2)] / (1 - FeO_2)$$

Where FR is flow rate (mL min^{-1}), FiO_2 and FeO_2 are fractional O_2 concentrations of incurrent and excurrent air, respectively, and $FiCO_2$ and $FeCO_2$ are fractional CO_2 concentrations. Mean metabolic rates were calculated from continuous recordings (1 s resolution) at each temperature step.

Statistical analyses

Analytical framework

To distinguish evolutionary (species-level) from individual (within-species) sources of torpor expression, M_b was partitioned into between-species and within species components: (1) the species mean M_b (between-species component), representing differences in M_b among species, and (2) the deviation of each individual from its species mean (within-species component), representing individual variation within species. This decomposition allowed us to evaluate whether the different physiological responses were primarily associated with interspecific or intraspecific variation in M_b, while accounting for energetic state and phylogenetic relationships. Body mass components were standardized (z-transformed) prior to analyses. Each response variable was analyzed separately because torpor use (binary), torpor depth (continuous), and normothermic metabolic rate represent distinct physiological responses.

Euthermic traits

To estimate *BMR* and critical temperatures, we fitted linear regressions using the "lm" function (package "stats"), with T_a as the predictor and metabolic rate as the response. Breakpoints were identified using the "davies.test" function from the "segmented" package (Muggeo 2008). The lower and upper breakpoints corresponded to T_{LC} and T_{UC}, respectively, and *BMR* was calculated as the mean metabolic rate within the thermoneutral zone (Medina-Bello et al. 2023; Ayala-Berdon J, Medina-Bello 2024).

Torpor use models

Torpid metabolic rate (mL O_2 h^{-1}) was defined as a sustained reduction in metabolic rate below both *BMR* and the expected values at T_{LC}, indicating transition from euthermia to torpor (Ayala-Berdon J, Medina-Bello 2024). A sustained reduction was defined as a decrease maintained for at least one full recording interval (≥ 5 min). Torpor use was classified when this pattern was consistently observed during cooling under each experimental treatment.

Torpor use was coded as a binary variable (1 = torpor, 0 = normothermy) and was analyzed using phylogenetic generalized linear mixed models implemented in the package “phyr”. Models were fitted with a binomial error distribution and logit link. The full model included the between- and within species M_b, treatment (energy-supplied vs. energy-restricted), and their interaction, as fixed effects. Individual identity was included as random effect, and phylogenetic relationship among species were incorporated as covariance structure using species-level random effect linked to the phylogenetic tree. To evaluate whether torpor propensity was primarily associated with species-level M_b, we additionally fitted a reduced model including only the between-species component and treatment. Because likelihood-based information criteria are not available for binomial phylogenetic mixed models in phyr, the importance of phylogeny was evaluated by (1) inspecting the variance of the phylogenetic random effect, and (2) comparing fixed-effect estimates with those from a non-phylogenetic generalized linear mixed model using “lme4”.

Torpor depth

Torpor depth was quantified as a continuous numeric torpid metabolic rate. To evaluate whether torpor depth was primarily associated with within-species variation in body mass, we fitted a phylogenetic linear mixed model (Gaussian error distribution) applied to log-transformed values. The model included the between- and within-species M_b, treatment, ambient temperature (T_a), and all interactions among within species M_b, treatment, and T_a. Ambient temperature was standardized (z-transformed). Individual identity was included as a random effect, and phylogenetic covariance among species was incorporated as described above.

Normothermic metabolic rate

To determine whether interspecific and intraspecific variation in M_b influenced euthermic energy expenditure, normothermic metabolic rate was analyzed using phylogenetic linear mixed models applied to log-transformed metabolic rate numeric values during euthermia. Analyses were restricted to observations in which individuals remained normothermic. Fixed effects included between- and within-species M_b, treatment, T_a, and their interactions. Individual identity and phylogenetic covariance were included as random effects.

Phylogenetic structure

Phylogenetic relationships among species were incorporated as a species random effect using a covariance matrix derived from the dated molecular phylogeny of bats, pruned to include only the study species. A negligible variance component was interpreted as lack of phylogenetic signal in the response variable.

Model diagnosis and visualization

Model assumptions were evaluated by visual inspection of residuals and fitted values. Continuous predictors were standardized to improve model convergence and interpretability. Because fixed-effect estimates were nearly identical between phylogenetic and non-phylogenetic models (see Results), predictions were generated from equivalent non-phylogenetic mixed models for visualization (lme4). Predictions were evaluated at mean values (zero after standardization) of covariates not shown on the axes. Slopes (β) were extracted from model summaries. All statistical tests were two-tailed, and significance was assessed at $\alpha = 0.05$.

Results

Torpor use

Torpor use was primarily explained by interspecific variation in body mass (M_b; 42 observations from 21 individuals under two energetic treatments; Table 1). Larger species exhibited a significantly greater probability of entering torpor than smaller species (Fig. 3). The probability of entering torpor increased significantly with the between-species component of M_b ($\beta = 1.48 \pm 0.54$ SE, $Z = 2.72$, $p = 0.006$). Each 1 SD increase in species mean M_b increased the odds of entering torpor by approximately 4.4-fold. Energy

restriction also tended to increase the probability of torpor (β = 1.33 ± 0.77 SE, Z = 1.73), corresponding to a ~ 3.8-fold increase in odds, although this effect was marginally non-significant (p = 0.083). In contrast, within species variation in M_b did not significantly affect torpor use (β = -1.15 ± 0.96 SE, Z = -1.20, p = 0.23), nor did its interaction with treatment (β = -0.90 ± 1.10 SE, Z = -0.82, p = 0.41). The variance associated with the phylogenetic random effect was negligible ($\sigma^2 \approx 9.0 \times 10^{-6}$) as were the species-level ($\sigma^2 \approx 3.3 \times 10^{-5}$) and individual-level random effects ($\sigma^2 \approx 8.0 \times 10^{-6}$), indicating that most variation was explained by fixed effects. Fixed-effect estimates were virtually identical between phylogenetic and non-phylogenetic models, confirming that phylogeny did not influence inference.

Table 1
Summary of sample sizes for each bat species. Values represent the total number of individuals per species (N) and total number of observations [obs], as well as the number of individuals in each metabolic state (n) and, in parentheses, the corresponding number of observations. During experimental trials, some individuals entered torpor or remained euthermic under different treatments (see Supplementary Material). Euthermic and torpor categories correspond to observations in which individuals remained normothermic or entered torpor, respectively. Because individuals were measured under both treatments, counts are not independent across treatments.

Bat species	**N [obs]**	**Treatment**	**Euthermic** ***n*** **(obs)**	**Torpor** ***n*** **(obs)**
Eptesicus fuscus	4 [16]	Energy-supplied	0 (0)	4 (16)
		Energy-restricted	1 (4)	3 (12)
Lasiurus cinereus	4 [16]	Energy-supplied	1 (4)	3 (12)
		Energy-restricted	0 (0)	4 (16)
Lasiurus ega	3 [12]	Energy-supplied	2 (8)	1 (4)
		Energy-restricted	1 (4)	2 (8)
Myotis californicus	2 [8]	Energy-supplied	2 (8)	0 (0)
		Energy-restricted	1 (4)	1 (4)
Myotis thysanodes	2 [8]	Energy-supplied	2 (8)	0 (0)
		Energy-restricted	2 (8)	0 (0)
Myotis volans	6 [24]	Energy-supplied	5 (20)	1 (4)
		Energy-restricted	2 (8)	4 (16)

Torpor depth

In contrast to torpor use, torpor depth, measured as log-transformed torpid metabolic rate, was primarily associated with within-species variation in M_b (n = 92 observations during torpor; Table 1). Within species, heavier individuals exhibited significantly higher torpid metabolic rates (β = 0.55 ± 0.21 SE, Z = 2.63, p = 0.0086), indicating shallower torpor states. Neither the between-species component of M_b (β = 0.03 ± 0.18 SE, Z = 0.19, p = 0.85) nor treatment alone (β = -0.23 ± 0.21 SE, Z = -1.35, p = 0.18) had significant effects. Ambient temperature (T_a) alone had no significant effect (β = -0.05 ± 0.11 SE, Z = -0.45, p = 0.65). However, there was a significant interaction between treatment and T_a (β = 0.30 ± 0.14 SE, Z = 2.09, p = 0.037), indicating that the effect of temperature on torpor depth differed between energy-supplied and energy-restricted individuals (Fig. 4). Phylogenetic ($\sigma^2 \approx 1.3 \times 10^{-6}$) and species-level variance ($\sigma^2 \approx 3.6 \times 10^{-6}$) were negligible, whereas individual-level variance was substantial ($\sigma^2 \approx 0.43$), indicating that torpor depth was structured at the individual level rather than by phylogeny.

Normothermic metabolic rate

Normothermic metabolic rate exhibited the same general pattern as torpor depth (76 observations from individuals which remained normothermic; Table 1). Within species, individuals with higher M_b relative to their species mean exhibited significantly higher metabolic rates (β = 0.32 ± 0.10 SE, Z = 3.14, p = 0.0017) (Fig. 5). In contrast, the between-species component of M_b had no significant effect (β = 0.18 ± 0.14 SE, Z = 1.29, p = 0.20). Neither treatment (β = 0.01 ± 0.18 SE, Z = 0.06, p = 0.95) nor T_a (β = 0.10 ± 0.09 SE, Z = 1.12, p = 0.26) influenced normothermic metabolic rate, and no significant interactions were detected. Despite the strong effect of within-species M_b, normothermic metabolic rate retained a substantial phylogenetic variance component ($\sigma^2 \approx 0.093$), together with moderate individual-level variance ($\sigma^2 \approx 0.075$), suggesting that baseline metabolic rates retain phylogenetic signal, even though fixed effects were primarily driven by within-species variation. Together these analyses indicate that torpor propensity was primarily structured by interspecific variation in M_b, whereas both torpor depth and normothermic metabolic rate were explained predominantly by intraspecific variation.

Discussion

Our study demonstrates that different components of torpor expression are governed at different biological levels. Whereas the probability of entering torpor was associated primarily with interspecific variation in body mass (M_b), both torpor depth and normothermic metabolic rate were explained by within-species variation. These contrasting patterns indicate that the decision to enter torpor and the regulation of metabolism during torpor represent distinct physiological processes. Furthermore, the negligible phylogenetic signal for torpor use and torpor depth suggests that these traits are evolutionarily labile and strongly influenced by ecological and physiological factors.

Contrary to the classical expectation that smaller endotherms should rely more heavily on torpor, we found that the probability of torpor use was higher in larger species compared to smaller ones. This pattern differs from that commonly reported across mammals, where smaller species are generally

more prone to use torpor due to their higher mass-specific metabolic rates and limited energy reserves (Körtner and Geiser 2000; Geiser 2008). Rather than contradicting this general pattern, our results suggest that the relationship between body size and torpor use depends on ecological context and the scale at which variation is evaluated. Unlike many small mammals, in which torpor is often employed as a last-resort response to energetic stress, its use in bats is widespread and may represent a routine energy-management strategy. For example, several vespertilionid bats have been observed to enter torpor rapidly upon reaching their lower critical temperature, as energetic demands increase with decreasing ambient temperature (T_a), suggesting an efficient energy-conserving response (Ayala-Berdon and Medina-Bello 2024). This difference may help explain the pattern observed here.

One possible explanation for the greater use of torpor in larger species is that larger absolute energy reserves facilitate active rewarming, thereby reducing the costs associated with torpor-arousal cycles (Geiser and Ruf 1995; Lovegrove et al. 1999). This capacity may allow larger individuals to use torpor more flexibly as a controlled energy-allocation mechanism rather than as an emergency response. In contrast, smaller bats may face a risk of failing to rewarm due to limited energy reserves, especially in temperate ecosystems as Santa Cruz Moxolahuac where ambient temperatures may decrease below 0 in the dry-cold season. Mathematical estimates suggest that, although animals reduce their metabolic rate by 5–30% below basal metabolic rate during torpor, net energetic savings are reduced by approximately 40–70% due to the costs of rewarming (Geiser and Ruf 1995; Lovegrove et al. 1999). Consistent with this interpretation, torpor use in our study was determined by interspecific variation in M_b, indicating that species-level energetic strategies play an important role in determining whether individuals enter torpor.

In contrast to the interspecific pattern observed for torpor use, both torpid and normothermic metabolic rates were positively related to intraspecific variation in M_b. This pattern is consistent with metabolic scaling, whereby larger individuals possess greater amounts of metabolically active tissue, resulting in higher absolute metabolic rates (Savage et al. 2004; Kozłowski et al. 2003; Farrell-Gray and Gotelli 2005; McNab 2019; Medina-Bello and Ayala-Berdon 2024). Within species, this scaling relationship is expected to generate higher metabolic rates in larger individuals, even under conditions of metabolic suppression such as torpor. Additionally, smaller bats may exhibit higher thermal conductance during torpor, facilitating greater heat loss and deeper reductions in metabolic rate (Geiser 2021). Together, these mechanisms likely explain why smaller individuals within species achieved lower torpid metabolic rates than larger individuals. Our results further indicate that variation in M_b within species provides a mechanism for fine-scale physiological adjustment among bats, allowing individuals to modulate energy expenditure in response to immediate energetic and environmental conditions. We also found that torpid metabolic rate was influenced by an interaction between energetic state and T_a, with the effect of temperature differing between energy-supplied and energy-restricted individuals. This suggests that the depth of torpor is not determined solely by M_b but is modulated by the interaction between environmental conditions and individual energetic state. Taken together, these findings indicate that the physiological processes underlying torpor initiation differ from those governing metabolic regulation

once torpor has been initiated. Entering torpor appears to reflect species-level energetic strategies, whereas the magnitude of metabolic suppression is adjusted primarily through individual physiological variation and environmental conditions.

Overall, our results indicate that, while the decision to enter torpor is structured at the species level, the magnitude of metabolic suppression is governed by individual-level physiological and environmental factors. This decoupling between torpor initiation and metabolic regulation suggests that torpor should not be viewed as a single physiological trait, but rather a sequence of physiological responses regulated by distinct mechanisms operating at different biological levels. Variation in torpor expression may therefore represent one mechanism through which coexisting species differ in their energetic responses to shared environmental conditions. Larger species may be more likely to enter torpor under energetically challenging conditions, whereas the extent of metabolic suppression appears to be regulated primarily by individual physiological condition and T_a. However, once in torpor, smaller individuals within species may reach lower torpid metabolic rates than larger individuals. Although the number of individuals per species was modest, the repeated-measures design and decomposition of M_b allowed us to distinguish consistent interspecific and intraspecific patterns across the study community. Whether these physiological differences ultimately translate into ecological partitioning and coexistence under natural conditions remains an important question for future studies.

Declarations

Supplementary Information

All data associated with this investigation are presented in the Supplementary Material.

Statement on the use of AI

AI-assisted technologies (ChatGPT) were used to correct the grammar and language of the manuscript text

Conflict of interest

Authors declare no conflict of interest.

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Author Contribution

J.A.B.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, software, supervision, visualization, writing—original draft, writing—review and editing; KIMB: conceptualization, data curation, investigation, methodology, writing—original draft, writing—review and editing.

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Data Availability

All data associated with this investigation are presented in the Supplementary Material.

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Figures

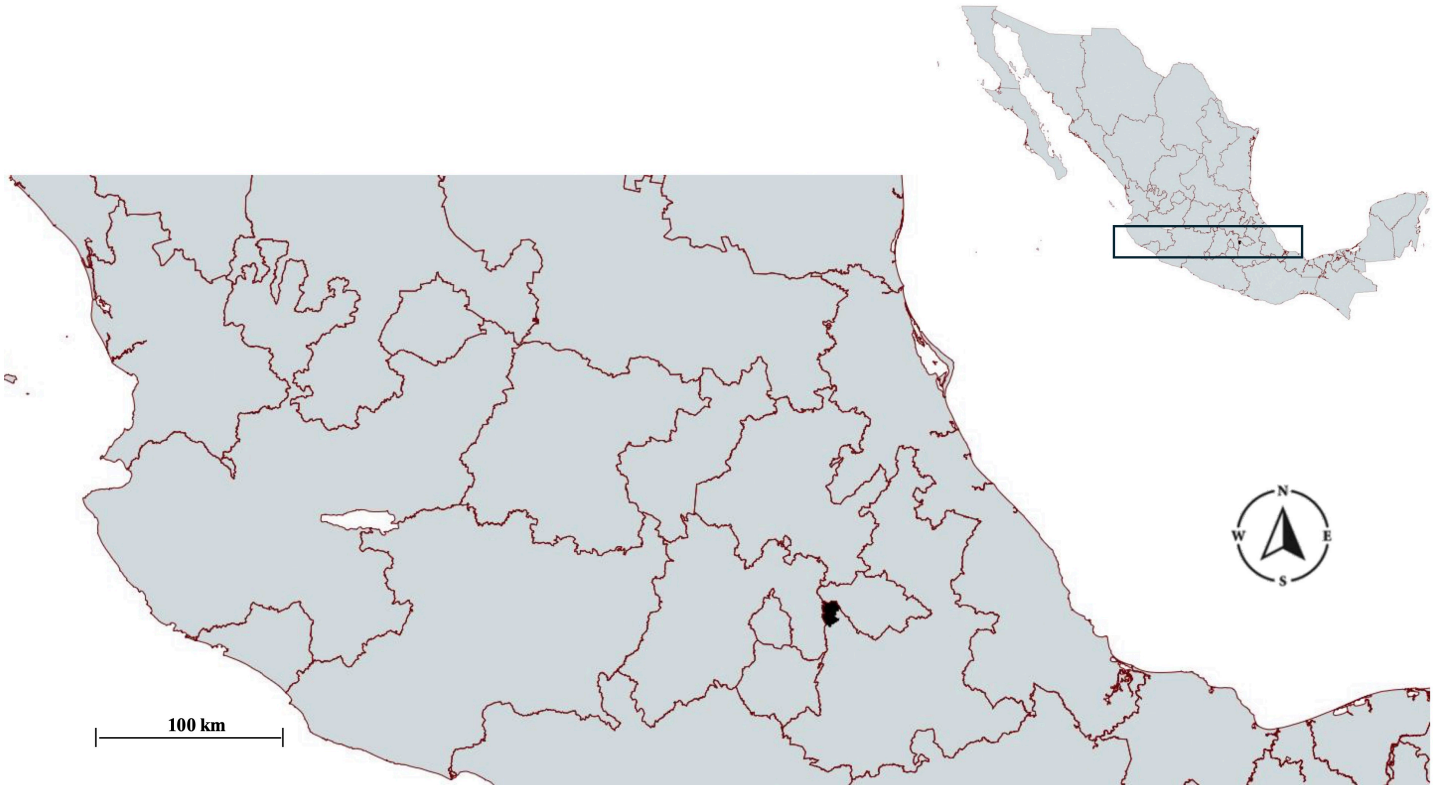


Figure 1

Location of the study site in Santa Cruz Moxolahuac, Puebla, central Mexico. The upper-rigth inset shows the location of the study region within Mexico, and the black marker indicates the study site. State bundaries and 100-Km scale bar are shown for geographic reference. The base map was generated using MapChart (https://www.mapchart.net) and edited with GIMP-2.10 (https://www.gimp.org).

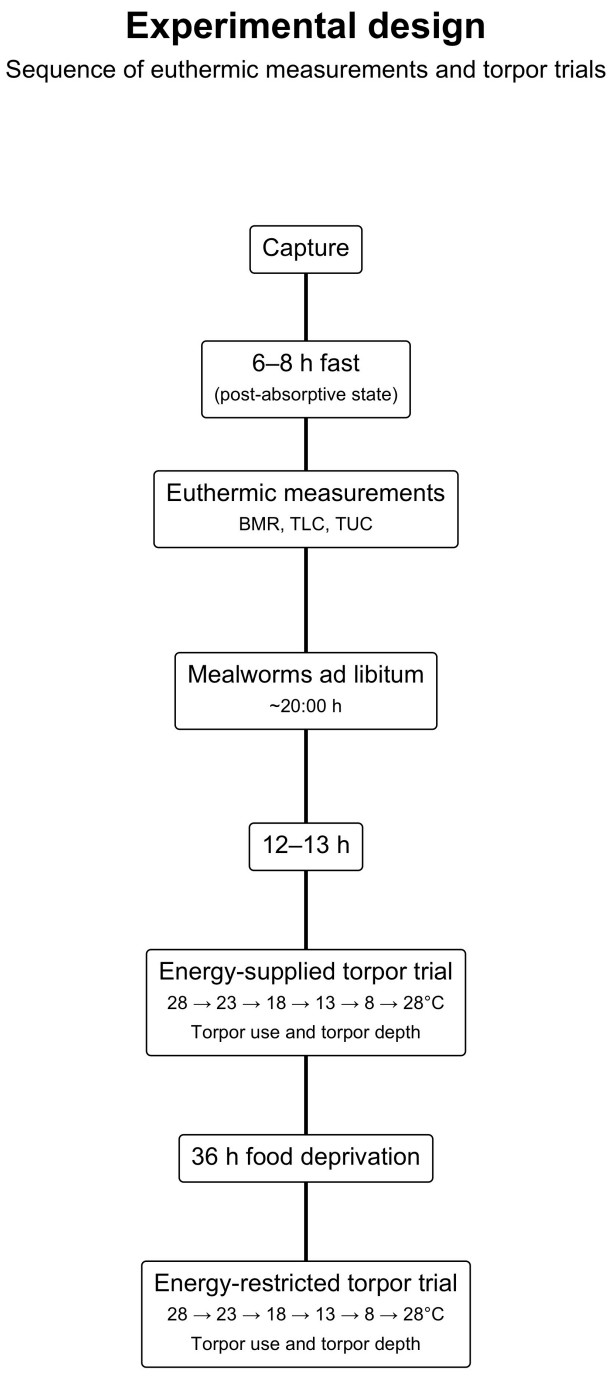


Figure 2

Experimental design showing the sequence of euthermic measurements and torpor trials. Following capture and a 6-8 h fasting period, basal metabolic rate (BMR) and the lower (T_{LC}) and upper (T_{UC}) critical temperatures were determined. Bats were subsequently fed mealworms *ad libitum* and, 12-13 h later, underwent the energy-supplied torpor trail. The same individuals were then subjected to 36 h of

food deprivation before the energy-restricted torpor trial. During both torpor trials, ambient temperature (T_a) was progressively decreased from 28 to 8 ºC and subsequently returned to 28 ºC.

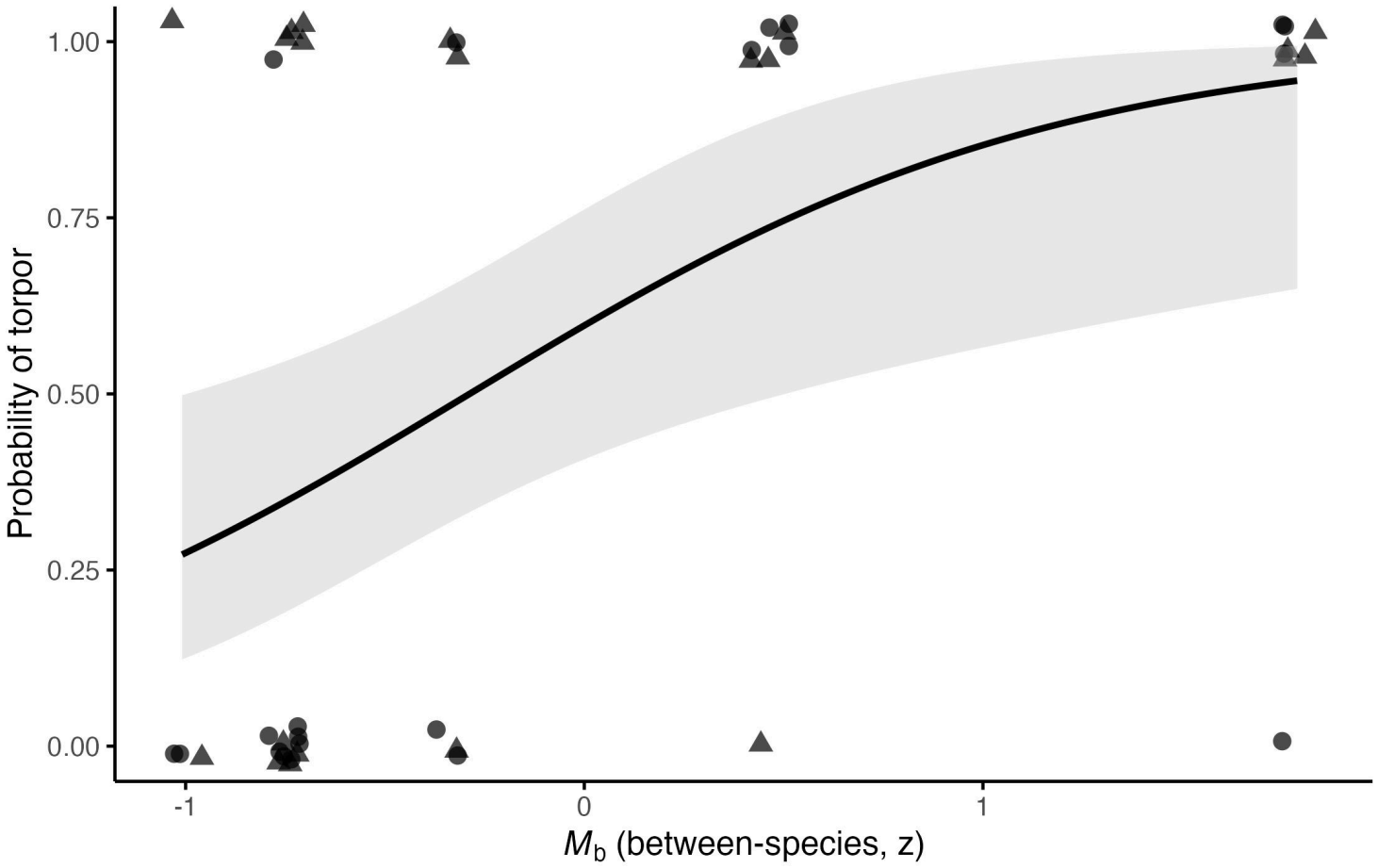


Figure 3

Relationship between the between-species component of body mass (M_b; z-transformed) and the probability of entering torpor. Points represent individual observations, with circles indicating energy-supplied bats and triangles indicating energy-restricted bats. The solid line shows the model-predicted probability of torpor, and the shaded area represents the 95 % confidence interval.

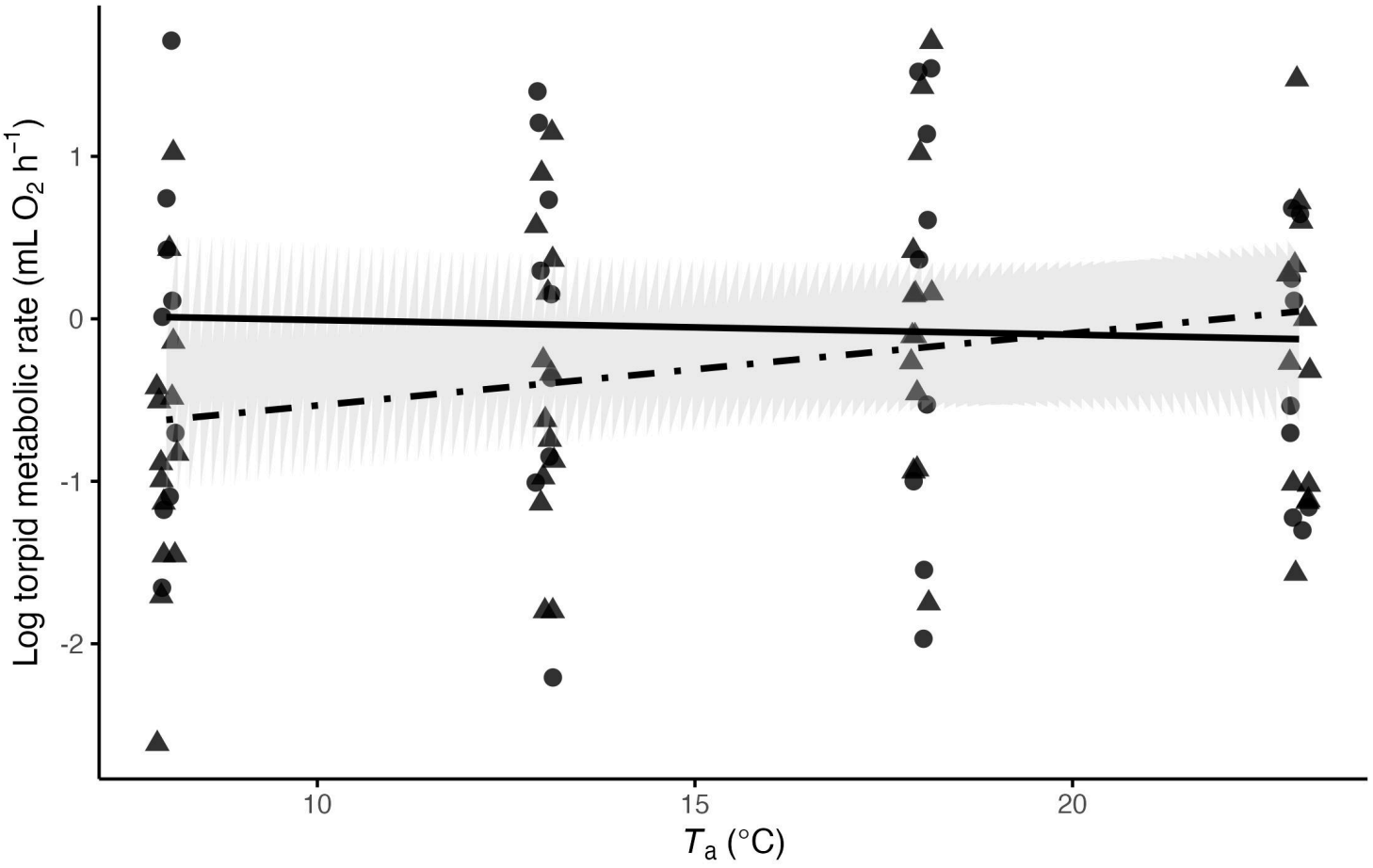


Figure 4

Relationship between ambient temperature (T_a) and torpor depth, measured as log-transformed torpid metabolic rate. Points represent individual observations, with circles indicating energy-supplied bats and triangles indicating energy-restricted bats. Lines show model-predicted values for energy-supplied (solid) and energy-restricted (dot-dash) individuals, evaluated at mean values of the between- and within-species components of body mass (M_b). Shaded areas represent 95% confidence intervals.

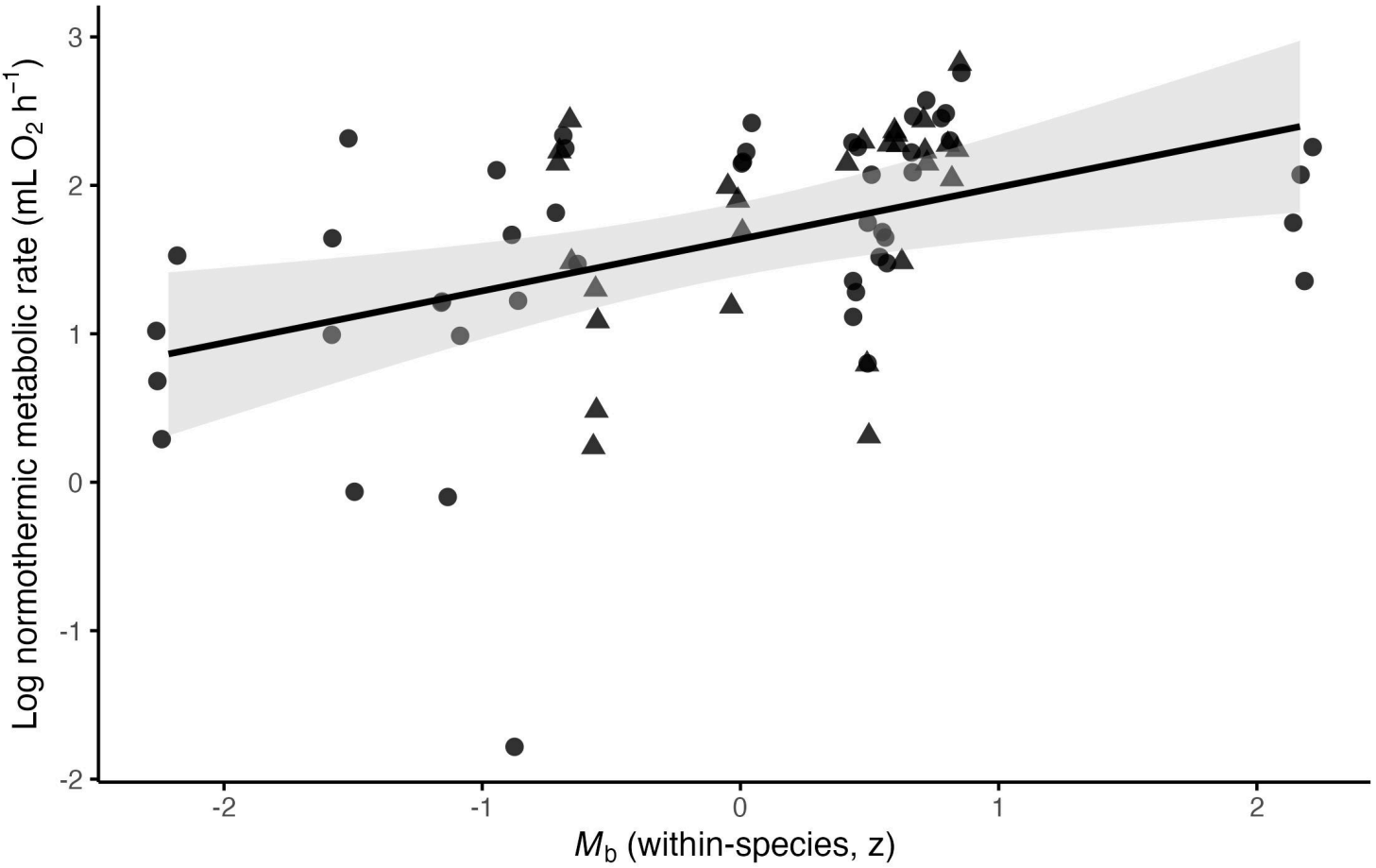


Figure 5

Relationship between the within-species component of body mass (M_b; z-transformed) and normothermic metabolic rate, measured as log-transformed oxygen consumption. Points represent individual observations, with circles indicating energy-supplied bats and triangles indicating energy-restricted bats. The solid line shows model-predicted values evaluated at mean values of the between-species component of M_b, and the shaded area represents the 95% confidence interval.

Supplementary Files

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- SupplementaryMaterial.txt