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When to initiate torpor use? Food availability times the transition to winter phenotype in a tropical heterotherm

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Abstract Timing of winter phenotype expression determines individual chances of survival until next reproductive season. Environmental cues triggering this seasonal phenotypic transition have been rarely investigated, although they play a central role in the compensation of climatic fluctuations via plastic phenotypic adjustments. Initiation of winter daily torpor use – a widespread energy saving phenotype – could be primarily timed according to anticipatory seasonal cues (anticipatory cues hypothesis), or flexibly fine-tuned according to actual energy availability (food shortage hypothesis). We conducted a food supplementation experiment on wild heterothermic primates (grey mouse lemurs, *Microcebus murinus*), at the transition to the food-limited dry season, i.e. the austral winter. As expected under the food shortage hypothesis, food supplemented individuals postponed the seasonal transition to normal torpor use by 1-2 month(s), spent 4 time less torpid, and exhibited minimal skin temperature 6°C higher than control animals. This study provides the first *in situ* experimental evidence that food availability, rather than abiotic cues, times the launching of torpor use. Fine-tuning of the timing of seasonal phenotypic transitions according to actual food shortage should provide heterotherms with a flexible adaptive mechanism to survive unexpected environmental fluctuations.

Key-words daily torpor, food supplementation, heterothermy, phenotypic plasticity, winter

Introduction

Understanding how individuals allocate energy between competing life history traits (Stearns 1992) and under changing environmental conditions (Visser et al. 2010; Lane et al. 2012) are key theoretical challenges in ecology. Organisms have to adjust the timing of major biological events requiring high energy expenditure to the seasonality of their habitats (Miller-Rushing et al. 2010). Much research has focused on assessing selection for the timing of reproduction, particularly impacts of climate change at uncoupling phenologies of resources and consumers (Miller-Rushing et al. 2010; Visser et al. 2010; Sheriff et al. 2011). But to reproduce, iteroparous organisms also need to survive the unfavourable season, i.e. winter (e.g., Patil et al. 2013). Hence, the first crucial seasonal transition is the switch to a winter phenotype, defined as the suite of phenotypic changes (including gene expression, physiology, anatomy, behaviour) that are expressed during the unfavourable season (Ozgul et al. 2010; Miller-Rushing et al. 2010; Helm et al. 2013).

Two main behavioural strategies have evolved to survive seasonal resource scarcity: migrate to more productive grounds or rely on energy saving mechanisms. Timing of phenological transitions has major fitness consequences (Thomas et al. 2001; Visser et al. 2010), and flexibility of this timing is a key response to climatic variability (Canale and Henry 2010; Reed et al. 2010). Yearly migration by birds is the most studied seasonal phenotypic change among vertebrates (Gienapp et al. 2007). Non-migratory animals reduce activity and fuel metabolic needs with stored energy reserves to match energy expenditure with energy availability. Heterothermic endotherms further reduce their daily energetic expenditure by entering a torpid state (Heldmaier et al. 2004; Geiser 2013), which increases survival (Turbill et al. 2011; Bieber et al. 2012). We refer to heterothermy as the faculty of some endotherms to undergo a controlled reduction of their body temperature during bouts of hibernation and daily torpor, in association with metabolism reduction (Heldmaier et al. 2004; Geiser 2013). Timing of switches to winter phenotype should be crucial for the chances of free-ranging heterotherms to survive and subsequently reproduce (Ozgul et al. 2010; Lane et al. 2012).

As heterothermy is generally expressed during harsh climatic conditions and/or lean periods (Körtner and Geiser 2000; Heldmaier et al. 2004; Vuarin and Henry 2014), it has commonly been regarded as an adaptive strategy to cope with energy shortage (Humphries et al. 2003a; Munro et al. 2005; Landry-Cuerrier et al. 2008; see Geiser 2013, Vuarin and Henry 2014, for other functions). But heterothermy comes with costs, such as oxidative stress, reduced immunocompetence or cognitive impairments (Humphries et al. 2003a; Angilletta et al. 2010). Hence, heterothermy use should be optimized according to current temperature and food constraints (Humphries et al. 2003a). For heterothermy to remain beneficial, organisms need to rely on environmental cues that allow them to accurately assess and anticipate forthcoming energetic constraints (Visser et al. 2010). A major risk when compensating environmental changes by phenotypic flexibility is to express a suboptimal phenotype, that does not maximize fitness in the new environment (Auld et al. 2010; Reed et al. 2010). When studying phenotypically flexible traits, it is therefore crucial to identify the primary environmental cues that allow animals to accurately fine-tune seasonal adjustments of their phenotype (Piersma and Drent 2003; Canale and Henry 2010; Reed et al. 2010; Helm et al. 2013).

To date, it remains controversial whether heterothermy is mainly expressed in anticipation of upcoming seasonal unfavourable conditions, and triggered by fixed seasonal cues, or mainly a flexible thermal strategy that fine-tunes energy expenditure according to short-term fluctuations in energy availability (Vuarin and Henry 2014; Williams et al. 2014). Photoperiod is an ubiquitous, proximal environmental cue for seasonal acclimation (Helm et al. 2013). But proximate energetic constraints also influence the regulation of heterothermy. Decreasing air temperatures enhance heterothermy expression (e.g., Heldmaier et al. 2004; Landry-Cuerrier et al. 2008; Vuarin et al. 2013; Helm et al. 2013), and food shortage triggers its expression in captive mammals (see Vuarin and Henry 2014 for a review). Whether free-ranging heterotherms adjust their seasonal transition to regular hibernation/daily torpor use primarily according to seasonal food shortage (food shortage hypothesis) *versus* to other seasonal cues, such as photoperiod and weather conditions (anticipatory cues hypothesis), has never been tested experimentally.

In the wild, food supplementation during periods of natural food shortage is the most direct experimental approach to test for the dependence of heterothermy on energy availability (Humphries et al. 2003b; Vuarin and Henry 2014). The aim of the present study was to provide the first test of a direct dependence of the launching of torpor use on seasonal food availability under natural conditions. We experimentally manipulated food availability and quantified torpor use of grey mouse lemurs (*Microcebus murinus*; SEM Fig. S1) at the transition between the wet season and the dry season. This primate inhabits dry deciduous forests of western and southern Madagascar, where it experiences severe water and food shortage during the dry season, i.e. austral winter (Dammhahn and Kappeler 2008a). Grey mouse lemurs prepare in advance to this unfavourable season by a suite of physiological modifications (e.g., Génin and Perret 2003; Giroud et al. 2008; Giroud et al. 2009; Canale et al. 2011), including fat deposition (Perret and Aujard 2001; Schmid 2001). Internal energy storage is complemented with torpor-based energy saving to maintain a favourable energy balance. Grey mouse lemurs enter torpor mostly on a daily basis (Schmid 2001; Fig. S3) with large inter-individual variation in its expression (Vuarin et al. 2013). Because they evolved in a highly seasonal environment, but with poorly predictable intra- and inter-annual fluctuations in resource availability (Dewar and Richard 2007), we predict that they directly cue in on local resource availability to time the launching of their winter phenotype (Dawson 2008; Canale and Henry 2010; Helm et al. 2013), i.e. the use of daily torpor during the dry season. Animals living in unpredictable environments are indeed expected to be more phenotypically flexible, and to rely more on proximate cues of resource constraints, than those from more predictable environments (Canale and Henry 2010; Florant and Healy 2012; Geiser 2013). Therefore, if food shortage is a major seasonal environmental constraint, we predict food supplemented individuals to postpone their transition to winter phenotype, i.e. to minimize torpor use relative to control individuals exposed to the seasonal, gradual reduction in food availability.

Materials and methods

Study site and animals

The study was conducted in the dry deciduous forest of Kirindy/CNFEREF (44°39’E, 20°03’S, 30-60 m above sea level) in western Madagascar between March and October 2010. The climate is highly seasonal, with a hot wet season from December to March and a cool dry season with little or no rain from April to November (Dammhahn and Kappeler 2008a). Throughout the experiment from April to June, mean T_a was 23.9 ± 7.4°C, and it rained only twice, before the start of the experiment (13.7 mm on 12-Mar and 2.2 mm on 13-Mar).

Grey mouse lemurs forage in overlapping home ranges and do not cache food (Dammhahn and Kappeler 2008b). Hence, our experimental design had to be based on the comparison of daily torpor patterns between a control plot (named N5) and a plot with food supplementation (named CS5). The two plots are ca. 2 km apart within a continuous forest and defined by boundaries of grid systems (500 x 500 m) of small foot trails at 25 m intervals (Dammhahn and Kappeler 2008a). We compared the two plots in terms of vegetation structure, food availability, air temperature, and grey mouse lemurs' condition and torpor use (ESM1, Table S1). The only notable difference was that, before the experiment, body condition of individuals from the control plot was 22% higher than in individuals from the food supplemented plot. Unfortunately, we do not have the necessary data to document the temporal covariation between body condition and torpor use throughout the experiment. Because food supplementation is expected to induce a rapid modification of body condition, body condition at capture is likely to be a poor predictor of body condition days and weeks later during the experiment. We still assessed the robustness of our results to initial differences in body condition between plots (ESM2). Because effects related to body condition were non-significant (Table S2), and because the sign, strength and significance of effects of the other variables remained similar after the addition of a body condition effect (Table S3), we are confident that the effect of food supplementation in our experimental design was not confounded by ecological or physiological differences between the two plots.

Study animals were captured between 27-Mar and 08-Apr (1-Apr ± 4 days), using 120 Sherman live traps baited with banana, opened at dusk and collected at dawn. Captured individuals were kept in their trap for the day. Upon first capture, individuals were briefly anesthetized (10 μl Ketanest 100), marked individually with subdermally implanted microtransponders (Trovan, Germany), and measured (including body mass and head width; Dammhahn and Kappeler 2008a; Vuarin et al. 2013). Forty-three adult (i.e. > 50 g) individuals, 19 from the control plot and 24 from the food supplemented plot, were equipped with collar-mounted temperature loggers (~3 g, Weetag Lite, AlphaMach, Sainte-Julie, Canada; Fig. S1), which recorded skin temperature (T_{sk}) every 10 minutes. T_{sk} is a reliable indicator of core body temperature, at least for the characterization of daily torpor use (Dausmann 2005). All captured individuals were released at their site of capture shortly before dusk on the same day. At the end of the food supplementation experiment, study animals were recaptured between 09-Jun and 03-Nov (12-Aug ± 60 days) to retrieve the temperature loggers. Twenty-one individuals still equipped with loggers were recaptured (8 loggers out of 21 were retrieved 13 ± 4 days (range: 9−19-Jun) before the end of the food supplementation to maximize recovery rates as some individuals had already lost their collars at the beginning of June). Twelve other individuals were recaptured but had lost their collars, and 10 individuals disappeared. T_{sk} data could be recovered from all loggers but one. Statistical analyses are based on 9 individuals from the control plot (5 females, 4 males) and 11 from the food supplemented plot (4 females, 7 males), for which T_{sk} was recorded over 68 ± 14 days (range: 15 to 76 days; daily sample sizes per plot are presented in Table S4).

Food supplementation design

Food supplementation started on 08-Apr and lasted until 23-Jun (77 days) (Table S4). In the food supplemented plot, food was provided daily at 12 feeding platforms (Fig. S2) that were distributed across the plot according to locations of mouse lemurs aggregates. Grey mouse lemurs mainly forage on fruits and invertebrates (Dammhahn and Kappeler

2008a). Food supplementation consisted of a mix of smashed banana, raisins and cat pellets (ca. 395 kJ per feeder) provided every second day, and only smashed banana (ca. 144 kJ per feeder) provided on the following day. Mean daily energetic provisioning was ca. 270 kJ.day^{-1} per feeder, which corresponds to daily energetic requirements of 3 to 4 individuals of 50 g body mass (Schmid and Ganzhorn 2009; Canale et al. 2011). Feeding platform's design ascertained that they were highly attractive, and safe, for grey mouse lemurs, while preventing access by larger animals (ESM Fig S2). Similar-sized species were unlikely to feed at the platforms either because the type of food provided did not match their diet (*Microgale* and *Geogale* tenrecs, tufted-tailed rats *Eliurus myoxinus*), or because they were inactive at that time of the year (fat-tailed dwarf lemurs, *Cheirogaleus medius*). For logistical reasons, food provisioning was interrupted for 3 consecutive nights every second week (17−19Apr, 01−03-May, 15−17-May, 29−31-May and 12−14-Jun; Table S4).

Characterization of torpor use

Continuous profiles of T_{sk} (see Tsk_rawdata; ESM) were used to compute three variables that quantify the daily use of torpor (see Dataset; ESM). First, we determined whether an individual entered torpor or not on a given day (i.e. individual probability to enter torpor: P_{torp}, binomial variable; Table S4). Animals were considered torpid when their T_{sk} dropped below 30°C (following Schmid 2001). When T_{sk} oscillated around 30°C (184 measures of 1357), we applied the rules presented in Fig. S3 to diagnose torpor bouts. Second, for each identified torpor event, we computed the duration of torpor bout (D_{torp}, in hours ± 0.1), which corresponds to the time spent below 30°C during a day. These definitions of P_{torp} and D_{torp} allowed testing for the separate effect of food supplementation on the probability to enter torpor (P_{torp}) and the amount of time allocated to torpor by a torpid individual. Third, we also analysed minimal skin temperature ($T_{\mathrm{sk\ min}}$, °C), which objectively quantifies hypothermia without relying on a threshold temperature. During torpor bouts, $T_{\mathrm{sk\ min}}$ quantifies torpor depth (Vuarin et al. 2013).

Daily torpor use strongly depends on air temperature: the lower T_{a} during the resting phase, the longer and the deeper torpor bouts (Heldmaier et al. 2004; Geiser 2013; Vuarin et al. 2013). To adjust fluctuations in daily torpor use for temporal fluctuations in T_{a}, we recorded T_{a} at resting sites in both plots. As grey mouse lemurs generally rest in tree holes (Schmid 1998), we placed a temperature logger (iButtons, Maxim Integrated, USA) in four tree holes per plot (as in Vuarin et al. 2013). T_{a} was recorded every 20 min throughout the study period. We considered daily minimum T_{a} ($T_{\mathrm{a\ min}}$) in tree holes as a reliable index of the thermal constraint faced by resting animals, since $T_{\mathrm{sk\ min}}$ is achieved during the coldest hours of the day (i.e., when T_{a} is the lowest; Schmid 2001; Vuarin et al. 2013). Daily $T_{\mathrm{a\ min}}$ values for each plot were averaged across the four corresponding temperature loggers. Thermal conditions were similar and highly correlated between the two plots (Fig. 1; Table S1; Pearson's $R = 0.92$, $p < 0.01$).

Statistical analyses

As we recorded temporally repeated measures of P_{torp}, D_{torp} and $T_{\mathrm{sk\ min}}$ for the same individuals, we applied mixed effects models with individual identity entered as a random intercept to account for the within-individual non-independence among data points.

First, we tested whether torpor use (P_{torp}, $T_{\mathrm{sk\ min}}$) differed between the control and food supplemented plots over the nine days preceding the experiment (9 and 7 individuals, respectively). Difference in D_{torp} could not be tested for since only a single individual used torpor once in the food supplemented plot. Second, we tested our predictions about the negative effect of food supplementation on torpor use by analysing P_{torp}, D_{torp} and T_{sk}

$_{\mathrm{min}}$ data collected after the start of the experiment, from 09-Apr to 23-Jun. P_{torp} data were analysed with generalized linear mixed effects models fitted with a binomial distribution, using the 'lmer' function of 'lme4' package (v. 0.999999-2) for R, whereas both D_{torp} and $T_{\mathrm{sk\ min}}$ data were analysed using linear mixed effects models using the 'lme' function of 'nlme' package (v. 3.1-109) for R. We adjusted torpor variables for the additive fixed effects of time (assuming a linear effect of the number of days since the start of the experiment with 09-Apr set to 1; temporal patterns other than linearity did not receive a better statistical support), sex and daily $T_{\mathrm{a\ min}}$, and their interactions (time and sex, time and $T_{\mathrm{a\ min}}$, $T_{\mathrm{a\ min}}$ and sex). These variables influence daily torpor use: (1) low T_{a} is associated with longer and deeper torpor bouts, (2) female grey mouse lemurs are less active in the dry season and, thus, might express more torpor than males (Schmid and Kappeler 1998; Kraus et al. 2008; Dammhahn and Kappeler 2008b; Terrien et al. 2010), and (3) torpor is maximal during the height of the dry season (Schmid 2001).

To test our predictions, we added the fixed effect of experimental treatment (natural food availability, named 'control' *versus* food supplementation, named 'food supplemented'), and its interactions with other fixed effects: (1) between treatment and time to account for a potential change of the effect of treatment throughout the study period, (2) between treatment and sex because females are more prone to use daily torpor, and therefore might show a stronger response to the treatment than males, and (3) between treatment and $T_{\mathrm{a\ min}}$ as the effect of treatment could be countered by low T_{a}. Finally, we also tested for an interaction (1) between treatment, time and sex effects because the sex-dependence of the response to the treatment could change over time, and (2) between time, sex and $T_{\mathrm{a\ min}}$ effects because the sex-dependence of the sensitivity to T_{a} could change over time. To account for remaining non-independence between data points due to temporal autocorrelation, we also parameterized an auto-regressive structure of order 1 (‘corAR1’ function) for temporal dependence between residuals in the analyses of D_{torp} and $T_{\mathrm{sk\ min}}$ (see Vuarin et al. 2013 for details). For the treatment effect, the control plot was defined as intercept.

Final models were obtained after removal of all non-significant interactions and additive effects based on likelihood ratio tests between nested models. We proceeded in three steps: assessment of third order interactions, then of second order interactions and, eventually, of additive effects in presence of significant, non-nested interactions. At each step, the most parameterized model included all terms, so that each effect was assessed with a model adjusted for all others. The significance level was set to $p < 0.05$. We checked whether residuals of each final model followed a Gaussian distribution. Slopes of effects (β), and their standard errors (SE), are presented for full models and final models to allow assessment of the effects to the potential weaknesses of downward, stepwise model selection. Torpor parameter estimates are presented as mean ± standard deviation (SD).

Results

Over the nine days before the start of the experiment, torpor parameters did not differ between the two plots (respectively for P_{torp}, and $T_{\mathrm{sk\ min}}$: $\chi^2_1 = 0.74$, $p = 0.39$, $\beta = 1.75 \pm 2.55$ and $\chi^2_1 = 0.78$, $p = 0.38$, $\beta = -0.50 \pm 0.62$°C). The percentage of individuals entering torpor on a given day (P_{torp}) was 12.5% in the control plot and 3.2% in the food supplemented one (respectively, 3 of 9, and 1 of 7 individuals entered torpor at least once). $T_{\mathrm{sk\ min}}$ was 30.6 ± 1.6°C in the control plot and 31.1 ± 1.0°C in the food supplemented one. D_{torp} was 1.5 ± 1.3 h in the control plot and 0.5 h in the food supplemented plot for the single individual that used torpor once.

After the start of food supplementation, torpor use rapidly differed between the two plots (Fig. 2). P_{torp} was much lower in the food-supplemented plot than in the control one (Table 1). On average throughout the experiment, control individuals expressed torpor on 93.4% of the days sampled (N = 672 individual days), whereas food-supplemented individuals expressed torpor on only 27.5% of the days sampled (N = 685 individual days). These results are adjusted for the effects of time and $T_{\mathrm{a\ min}}$, with a general trend for increasing torpor use throughout the experiment, which was reinforced by low $T_{\mathrm{a\ min}}$ (time x $T_{\mathrm{a\ min}}$ interaction; Table 1), and for time and sex, with males increasing their use of torpor later than females (time x sex interaction; Table 1) (Fig. 2). However, the response to the food treatment did not vary through time (treatment x time interaction, Table 1), and did not differ according to sex (treatment x sex interaction, Table 1). The response was not reinforced by low T_{a} either (treatment x $T_{\mathrm{a\ min}}$ interaction, Table 1).

Among individuals using torpor, food supplementation reduced D_{torp}: control individuals spent more time torpid (12.7 ± 7.2 h) than food supplemented ones (3.3 ± 6.4 h; Table 1; Fig. 3A). Torpor duration increased through time and increased with decreasing $T_{\mathrm{a\ min}}$ (Table 1), regardless the food treatment (treatment x time and treatment x $T_{\mathrm{a\ min}}$ interactions; Table 1). This sensitivity of torpor duration to $T_{\mathrm{a\ min}}$ decreased throughout the season (time x $T_{\mathrm{a\ min}}$ interaction, Table 1). Males exhibited shorter torpor bouts than females (1.8 ± 5.0 *versus* 6.3 ± 7.7 h for the food supplemented plot and 11.6 ± 7.3 *versus* 13.6 ± 7.1 h for the control plot; Table 1), regardless the food treatment (treatment x sex interaction; Table 1).

Grey mouse lemurs from the food-supplemented plot maintained higher $T_{\mathrm{sk\ min}}$ during rest than those from the control plot (Table 1). Control individuals exhibited an average $T_{\mathrm{sk\ min}}$ of 20.7 ± 4.9°C whereas $T_{\mathrm{sk\ min}}$ of supplemented ones was 26.7 ± 5.8°C. This was no longer the case at the end of the experiment when food-supplemented females reached values similar to control females, while food-supplemented males still exhibited higher $T_{\mathrm{sk\ min}}$ than control males (treatment x time x sex interaction; Table 1; Fig. 3B). There was a general trend for decreasing $T_{\mathrm{sk\ min}}$ with time (i.e. increasing torpor depth; Table 1). Furthermore, $T_{\mathrm{sk\ min}}$ decreased with decreasing $T_{\mathrm{a\ min}}$ (Table 1), and this sensitivity of $T_{\mathrm{sk\ min}}$ to $T_{\mathrm{a\ min}}$ increased throughout the season (time x $T_{\mathrm{a\ min}}$ interaction, Table 1). Finally, males were less responsive to $T_{\mathrm{a\ min}}$ than females ($T_{\mathrm{a\ min}}$ x sex interaction, Table 1).

Discussion

This food supplementation experiment demonstrated that the increasing use of torpor at the transition between the wet season and the dry season is flexible, and adjusted to actual food availability in a free-ranging heterotherm. Individuals with access to supplementary food reduced torpor use, duration and depth. These results demonstrate that the timing of winter phenotype expression is fine-tuned according to energy availability, superseding the influence of abiotic cues of seasonal transition, such as photoperiod and climatic conditions. Nonetheless, daily torpor flexibility proved to be limited to the first months of the transition to the dry season. One to two months later than control individuals, food supplemented ones also switched to a winter phenotype, with regular daily torpor, despite continuous provisioning of supplementary food.

As predicted, grey mouse lemurs flexibly adjusted their use of torpor according to food availability, supporting the torpor optimization hypothesis (Humphries et al. 2003a), i.e. individuals use available energy to minimize heterothermy expression, which in turn reduces associated costs (Humphries et al. 2003a; Angilletta et al. 2010). The two other experiments of food supplementation with free-ranging heterotherms also support a proximate role of food availability in heterothermy regulation (Humphries et al. 2003b; Munro et al. 2005). They were both conducted with a temperate hibernator, the eastern chipmunk (*Tamias striatus*) during winter. Chipmunks rely on external energy storage (hoard of seeds) and on prolonged bouts of hypometabolism to survive the 4-7 months cold, boreal winter. Individuals with experimentally enlarged food stores spent more time euthermic and exhibited shallower torpor bouts (Humphries et al. 2003b; Munro et al. 2005). Six other studies support a proximate role of food availability in heterothermy regulation in free-ranging animals, despite major differences in ecological and energetic constraints operating in the different systems (Vuarin and Henry 2014). They either highlighted correlative evidence of the dependence of heterothermy use on food availability under natural conditions, experimentally manipulated food availability under semi-natural conditions, or indirectly induced food shortage through an experimental increase of intraspecific competition for food. One compelling correlative study showed that the probability to use torpor in Australian owlet-nightjars (*Aegotheles cristatus*) depended primarily on insect availability, and to a lesser extent on rainfall, with no influence of low air temperature (Doucette et al. 2012).

A key assumption of our experimental design was that natural food availability decreases during the transition to the dry season. Monitoring of insect biomass and plant phenology supported the assumption that fleshy fruit availability and flying insect abundance decrease to a minimal level during the heart of the dry season (Dammhahn and Kappeler 2008b). During our experiment, meteorological conditions corresponded to a typical start of the dry season (Schmid and Kappeler 1998; Dammhahn and Kappeler 2008b), with an abrupt stop of rainfall and decreasing night temperatures (Fig. 1), simultaneously with a typical, major increase of the proportion of animals using torpor in the control plot (Fig. 2; e.g., Schmid 2001). Overall, meteorological conditions and heterothermic responses of control individuals demonstrate that our experiment actually took place at the ecological transition between wet and dry seasons.

If food availability was the only determinant of torpor use, food supplemented individuals should have maintained their summer phenotype (i.e., normothermia) throughout the experiment. However, they started to regularly enter torpor after one (for females) to two (for males) months of food supplementation, and most individuals entered torpor by the end of the experiment (end of June; Fig. 2). Three non-mutually exclusive hypotheses could explain these temporal dynamics of the effect of food supplementation on torpor use. First, the influence of anticipatory seasonal cues (photoperiod) on torpor regulation may increase through time, as the predictable risk of resource shortage increases. At the onset of the dry season, daily torpor would be flexibly adjusted to proximal environmental cues reflecting actual ecological conditions. Later on, the influence of photoperiod would take over that of proximal cues of resource constraints to guarantee that individuals enter into the adaptive physiological and behavioural states that maximize survival chances to the predictable, harshest part of the dry season. The existence of such a time window of reduced influence of photoperiod relative to proximal cues during the transition between the favourable and the unfavourable seasons, i.e. when day length is a poor predictor of actual energetic constraints, has already been suggested in other heterotherms (Geiser 2004; Munro et al. 2005; Sheriff et al. 2012). It also allows birds to fine-tune the timing of spring migration (Cornelius et al. 2013) and reproduction initiation according to local conditions (Lambrechts and Perret 2000; Dawson 2008). Moreover, Paul et al. (2009) experimentally demonstrated in captive Siberian hamsters (*Phodopus sungorus*) that non-photic cues (food availability, social structure) inhibit the reproductive axis under autumn photoperiod, but not under summer photoperiod. Overall, these observations suggest that, during seasonal transitions, phenotypic change is facultative and largely influenced by proximal cues of environmental constraints, whereas it becomes obligate and mainly driven by photoperiod when the animals enters the height of the harsh season (winter/dry season). Second, torpor use may be regulated according to the size of internal fat stores, rather than directly by food availability. Anticipatory fattening is a prerequisite for sustained regular daily torpor and hibernation (Körtner and Geiser 2000). And individuals with high body condition are more prone to use daily torpor, and use it more flexibly, than lean individuals (Vuarin et al. 2013). Grey mouse lemurs would have used supplementary food to constitute larger fat reserves than usual, postponing their physiological shift to winter phenotype. According to the asset protection principle (Dammhahn 2012), food-supplemented animals would have initiated regular daily torpor use when the prospect of supplementary fat storage was offset by the costs of additional food acquisition (e.g., predation; Humphries et al. 2003a; Sheriff et al. 2011; Turbill et al. 2011; Bieber et al. 2014). The first bouts of daily torpor may have even increased the efficiency of conversion of supplemented food into fat stores (Sheriff et al. 2013a; Giroud et al. 2014). Third, natural food availability (Dammhahn and Kappeler 2008a) is expected to decrease through time. Grey mouse lemurs may have remained normothermic as long as natural food availability, supplemented by artificial food provisioning, was sufficient to fulfil their daily energetic requirements. But when natural food supplies were exhausted, individuals would have started to use daily torpor to counterbalance growing energetic deficits associated with insufficient supply of artificial food.

In grey mouse lemurs, as in other heterotherms (like eastern chipmunks or arctic ground squirrels *Urocitellus parryii*; Munro et al. 2005; Sheriff et al. 2011, 2013b), males use less daily torpor than females. They remain more active and can be captured throughout the dry season (Schmid and Kappeler 1998; Kraus et al. 2008; Dammhahn and Kappeler 2008b; Dammhahn and Kappeler 2013). Our results clarify this sex difference in daily torpor use: at the heart of the dry season (June), males expressed daily torpor as much as females in the control plot, although less than females in the food supplemented plot (Figs 2-3). The main difference is that males begun regular daily torpor use one month later than females (Fig. 2), and exhibited higher minimal skin temperatures throughout the experiment than supplemented females, who ended up exhibiting similar patterns than control individuals (Fig. 3). These sex-specific differences in seasonal timing of daily torpor use might result from reduced body condition of males relative to females during the dry season (Schmid and Kappeler 1998), and associated reduced propensity to use daily torpor (Kobbe et al. 2011; Vuarin et al. 2013), or from sex-differences in energetic requirements to engage in reproduction just after the dry season (Schmid and Kappeler 1998; Canale et al. 2012; see also Munro et al. 2005; Sheriff et al. 2013b). Our study is the first to document, in free-ranging grey mouse lemurs, that sex differences in activity patterns (Rasoazanabary 2006) result from the fact that males and females do not start using daily torpor at the same period of the dry season, males entering daily torpor later than females.

To conclude, the transition to winter phenotype is plastic and not primarily timed according to abiotic cues that allow the anticipation of the dry season. During the transition between wet and dry seasons, the seasonal transition to winter phenotype is fine-tuned according to food availability. The cues used to time the final, food-independent shift to the full, winter phenotype remain to be identified. It could be photoperiod, which would override the influence of current environmental conditions. In our changing world, identification of the cues that time heterothermy is an obligate preliminary step to determine which, and how, heterothermic animals could be resilient to human-induced disturbances of environmental cycles, like habitat destruction or climate change (Canale and Henry 2010; Lane et al. 2012; Geiser 2013; Williams et al. 2014).

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Table 1 Effects of treatment, minimal air temperature ($T_{\mathrm{a\ min}}$), time, sex, and their interactions on the probability to enter torpor on a given day (P_{torp}), torpor duration (D_{torp}, for torpid animals only) and minimal body temperature ($T_{\mathrm{sk\ min}}$), for 20 free-ranging grey mouse lemurs (*Microcebus murinus*).

	P_{torp}								D_{torp}								$T_{\mathrm{sk\ min}}$							
	Full model				**Final model**				**Full model**				**Final model**				**Full model**				**Final model**			
	β	**SE**	**Z value**	P	β	**SE**	χ^2	p	β	**SE**	**t-value**	P	β	**SE**	χ^2	p	β	**SE**	**t-value**	P	β	**SE**	χ^2	p
Intercept	23.99	7.71	3.11	**<0.01**	21.53	3.64			21.46	3.69	5.81	**<0.01**	19.54	2.85			10.55	2.29	4.61	**<0.01**	9.91	1.95		
Treatment	-13.56	6.06	-2.24	**0.03**	-10.61	1.56	27.23	**<0.01**	-8.81	3.69	-2.39	**0.03**	-8.31	1.98	14.24	**<0.01**	9.00	2.01	4.49	**<0.01**	8.93	2.09	17.71	**<0.01**
$T_{\mathrm{a\ min}}$	-1.19	0.34	-3.48	**<0.01**	-1.03	0.16	49.77	**<0.01**	-0.83	0.16	-5.17	**<0.01**	-0.67	0.12	55.33	**<0.1**	0.71	0.10	7.30	**<0.01**	0.74	0.08	460.6	**<0.01**
Time	0.13	0.25	0.50	0.62	-0.01	0.07	255.44	**<0.01**	0.03	0.07	0.45	0.65	0.07	0.05	71.55	**<0.01**	-0.15	0.04	-3.46	**<0.01**	-0.13	0.03	48.71	**<0.01**
Sex	2.03	9.46	0.21	0.83	0.45	1.59	9.97	**0.05**	-6.43	5.51	-1.17	0.26	-4.52	1.98	5.46	**0.02**	5.70	3.12	1.83	0.09	6.98	2.09	4.91	**0.03**
Treatment x Time	-0.09	0.10	-0.86	0.39			0.6	0.44	0.05	0.05	0.99	0.32			1.77	0.18	-0.11	0.03	-3.92	**<0.01**	-0.11	0.03	1.22	0.27
Treatment x Sex	-4.10	3.65	-1.12	0.26			0.33	0.56	-5.28	4.71	-1.12	0.28			2.18	0.14	-2.51	2.11	-1.19	0.25	-2.52	2.27	3.07	0.08
Treatment x $T_{\mathrm{a\ min}}$	0.24	0.27	0.89	0.37			0.61	0.44	0.06	0.10	0.58	0.56			0.35	0.56	-0.11	0.06	-1.93	**0.05**	-0.11	0.06	3.29	0.07
Time x $T_{\mathrm{a\ min}}$	0.01	0.01	1.10	0.27	0.01	0.003	6.57	**0.01**	0.01	0.003	2.93	**<0.01**	0.01	0.002	9.54	**<0.01**	0.004	0.002	1.98	**0.05**	0.003	0.001	5.92	**0.02**
Time x Sex	-0.31	0.26	-1.18	0.24	-0.16	0.04	17.8	**<0.01**	0.06	0.10	0.62	0.53			0.42	0.52	-0.002	0.06	-0.03	0.98	-0.03	0.03	3.37	0.07
$T_{\mathrm{a\ min}}$ x Sex	0.02	0.41	0.06	0.95			0.64	0.42	0.26	0.24	1.10	0.27			0.65	0.42	-0.21	0.13	-1.63	0.10	-0.27	0.06	21.18	**<0.01**
Treatment x Time x Sex	0.14	0.11	1.27	0.20			1.82	0.18	0.002	0.07	0.02	0.98			0.001	0.98	0.16	0.04	4.10	**<0.01**	0.16	0.04	14.97	**<0.01**
Time x $T_{\mathrm{a\ min}}$ x Sex	0.002	0.01	0.19	0.85			0.04	0.85	-0.004	0.01	-0.87	0.38			0.77	0.38	-0.001	0.003	-0.54	0.59			0.29	0.59

Figure legends

Fig. 1 Mean (± SD) minimal air temperature in tree holes for the control plot (light grey line) and the food supplemented plot (dark grey line) over the study period. Data were averaged across four temperature loggers in each plot.

Fig. 2 Observed (histogram) and predicted (dots) percentages of (a, b) control and (c, d) food supplemented male (♂) and female (♀) grey mouse lemurs (*Microcebus murinus*) entering torpor according to the date. White corresponds to normothermia and grey to torpor. Predicted values were estimated with the final model's parameters. "Control" refers to the control plot with natural food availability (4 males, 6 females) and "Food suppl." refers to the food supplemented plot (7 males, 4 females; daily sample sizes are reported in Table S4).

Fig. 3 Observed (± SD; dots) and predicted (lines) torpor bout duration for (a, b) control and (c, d) food supplemented male (♂) and female (♀) grey mouse lemurs according to the date (each dot corresponds to the mean ± SD of the duration for the day ± 2 days); and observed (± SD; dots) and predicted (lines) minimal skin temperature for (e, f) control and (g, h) food supplemented male (♂) and female (♀) grey mouse lemurs according to the date (each dot corresponds to the mean ± SD of the temperature for the day ± 2 days). Predicted durations and temperatures were estimated from the final model's parameters. "Control" refers to the control plot with natural food availability (4 males, 6 females) and "Food suppl." refers to the food supplemented plot (7 males, 4 females; Table S4).

Figure 1

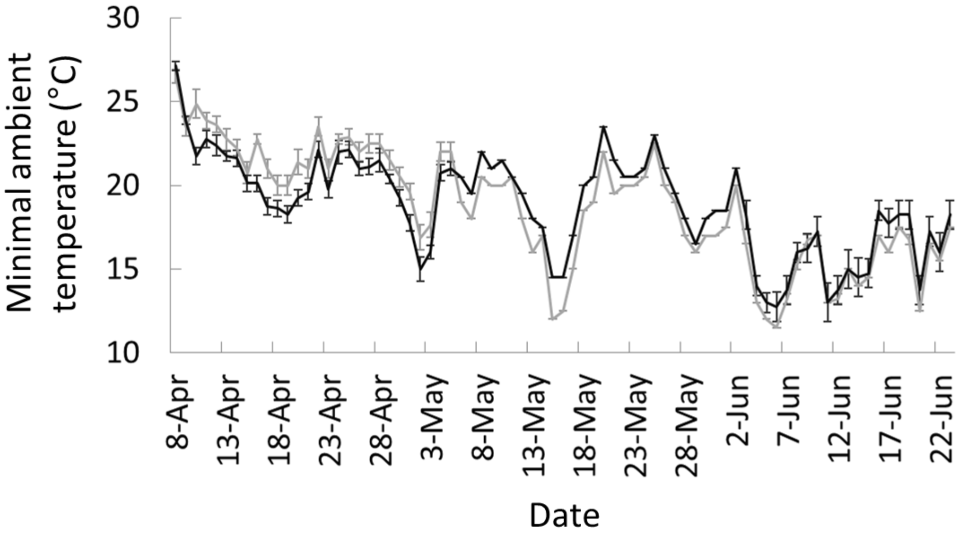

30
25
20
15
10
Minimal ambient temperature (°C)
8-Apr
13-Apr
18-Apr
23-Apr
28-Apr
3-May
8-May
13-May
18-May
23-May
28-May
2-Jun
7-Jun
12-Jun
17-Jun
22-Jun
Date

Figure 2

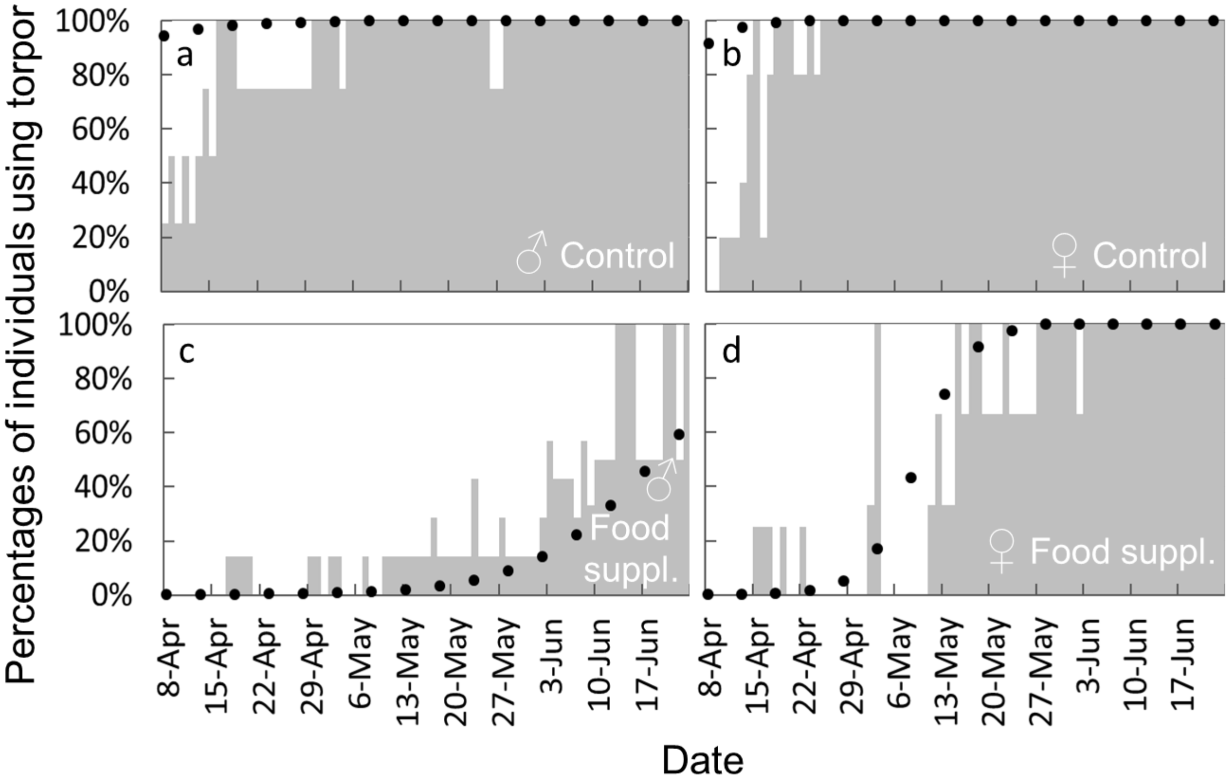
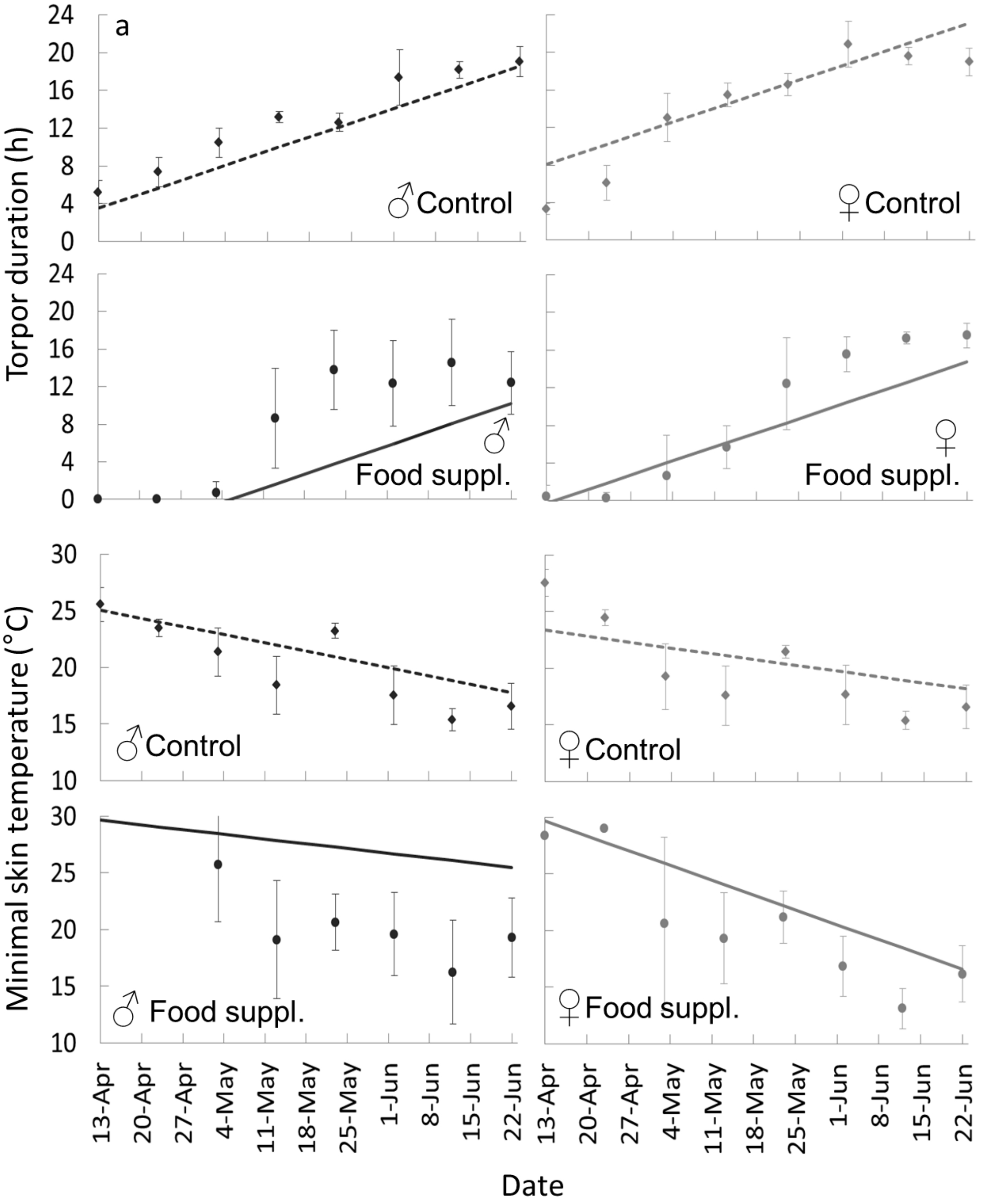

Percentages of individuals using torpor
100%
80%
60%
40%
20%
0%
a
♂ Control
b
♀ Control
c
♂ Food suppl.
d
♀ Food suppl.
8-Apr
15-Apr
22-Apr
29-Apr
6-May
13-May
20-May
27-May
3-Jun
10-Jun
17-Jun
Date

Figure 3


a
Torpor duration (h)
♂Control
♀Control
♂ Food suppl.
♀ Food suppl.
Minimal skin temperature (°C)
♂Control
♀Control
♂ Food suppl.
♀Food suppl.
0
4
8
12
16
20
24
10
15
20
25
30
13-Apr
20-Apr
27-Apr
4-May
11-May
18-May
25-May
1-Jun
8-Jun
15-Jun
22-Jun
Date