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1	When to initiate torpor use? Food availability times the
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23 Abstract Timing of winter phenotype expression determines individual chances of 24 survival until next reproductive season. Environmental cues triggering this seasonal 25 phenotypic transition have been rarely investigated, although they play a central role in 26 the compensation of climatic fluctuations via plastic phenotypic adjustments. Initiation of 27 winter daily torpor use -a widespread energy saving phenotype - could be primarily 28 timed according to anticipatory seasonal cues (anticipatory cues hypothesis), or flexibly 29 fine-tuned according to actual energy availability (food shortage hypothesis). We 30 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. 31 32 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 33 34 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, 35 36 rather than abiotic cues, times the launching of torpor use. Fine-tuning of the timing of 37 seasonal phenotypic transitions according to actual food shortage should provide 38 heterotherms with a flexible adaptive mechanism to survive unexpected environmental fluctuations. 39

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

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44 Introduction

45 Understanding how individuals allocate energy between competing life history traits (Stearns 1992) and under changing environmental conditions (Visser et al. 2010; Lane et 46 47 al. 2012) are key theoretical challenges in ecology. Organisms have to adjust the timing 48 of major biological events requiring high energy expenditure to the seasonality of their 49 habitats (Miller-Rushing et al. 2010). Much research has focused on assessing selection 50 for the timing of reproduction, particularly impacts of climate change at uncoupling 51 phenologies of resources and consumers (Miller-Rushing et al. 2010; Visser et al. 2010; 52 Sheriff et al. 2011). But to reproduce, iteroparous organisms also need to survive the 53 unfavourable season, i.e. winter (e.g., Patil et al. 2013). Hence, the first crucial seasonal 54 transition is the switch to a winter phenotype, defined as the suite of phenotypic changes 55 (including gene expression, physiology, anatomy, behaviour) that are expressed during 56 the unfavourable season (Ozgul et al. 2010; Miller-Rushing et al. 2010; Helm et al. 57 2013).

58 Two main behavioural strategies have evolved to survive seasonal resource scarcity: 59 migrate to more productive grounds or rely on energy saving mechanisms. Timing of 60 phenological transitions has major fitness consequences (Thomas et al. 2001; Visser et al. 61 2010), and flexibility of this timing is a key response to climatic variability (Canale and 62 Henry 2010; Reed et al. 2010). Yearly migration by birds is the most studied seasonal 63 phenotypic change among vertebrates (Gienapp et al. 2007). Non-migratory animals 64 reduce activity and fuel metabolic needs with stored energy reserves to match energy 65 expenditure with energy availability. Heterothermic endotherms further reduce their daily 66 energetic expenditure by entering a torpid state (Heldmaier et al. 2004; Geiser 2013), 67 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).

73 As heterothermy is generally expressed during harsh climatic conditions and/or lean 74 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 75 76 (Humphries et al. 2003a; Munro et al. 2005; Landry-Cuerrier et al. 2008; see Geiser 77 2013, Vuarin and Henry 2014, for other functions). But heterothermy comes with costs, 78 such as oxidative stress, reduced immunocompetence or cognitive impairments 79 (Humphries et al. 2003a; Angilletta et al. 2010). Hence, heterothermy use should be 80 optimized according to current temperature and food constraints (Humphries et al. 81 2003a). For heterothermy to remain beneficial, organisms need to rely on environmental 82 cues that allow them to accurately assess and anticipate forthcoming energetic constraints (Visser et al. 2010). A major risk when compensating environmental changes by 83 84 phenotypic flexibility is to express a suboptimal phenotype, that does not maximize 85 fitness in the new environment (Auld et al. 2010; Reed et al. 2010). When studying 86 phenotypically flexible traits, it is therefore crucial to identify the primary environmental 87 cues that allow animals to accurately fine-tune seasonal adjustments of their phenotype 88 (Piersma and Drent 2003; Canale and Henry 2010; Reed et al. 2010; Helm et al. 2013).

89 To date, it remains controversial whether heterothermy is mainly expressed in 90 anticipation of upcoming seasonal unfavourable conditions, and triggered by fixed 91 seasonal cues, or mainly a flexible thermal strategy that fine-tunes energy expenditure

92 according to short-term fluctuations in energy availability (Vuarin and Henry 2014; 93 Williams et al. 2014). Photoperiod is an ubiquitous, proximal environmental cue for seasonal acclimation (Helm et al. 2013). But proximate energetic constraints also 94 95 influence the regulation of heterothermy. Decreasing air temperatures enhance 96 heterothermy expression (e.g., Heldmaier et al. 2004; Landry-Cuerrier et al. 2008; Vuarin 97 et al. 2013; Helm et al. 2013), and food shortage triggers its expression in captive 98 mammals (see Vuarin and Henry 2014 for a review). Whether free-ranging heterotherms 99 adjust their seasonal transition to regular hibernation/daily torpor use primarily according 100 to seasonal food shortage (food shortage hypothesis) versus to other seasonal cues, such 101 as photoperiod and weather conditions (anticipatory cues hypothesis), has never been 102 tested experimentally.

103 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 104 105 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 106 107 seasonal food availability under natural conditions. We experimentally manipulated food 108 availability and quantified torpor use of grey mouse lemurs (Microcebus murinus; SEM 109 Fig. S1) at the transition between the wet season and the dry season. This primate inhabits 110 dry deciduous forests of western and southern Madagascar, where it experiences severe 111 water and food shortage during the dry season, i.e. austral winter (Dammhahn and 112 Kappeler 2008a). Grey mouse lemurs prepare in advance to this unfavourable season by a 113 suite of physiological modifications (e.g., Génin and Perret 2003; Giroud et al. 2008; 114 Giroud et al. 2009; Canale et al. 2011), including fat deposition (Perret and Aujard 2001; 115 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 116 117 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 118 119 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 120 121 the launching of their winter phenotype (Dawson 2008; Canale and Henry 2010; Helm et 122 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 123 124 to rely more on proximate cues of resource constraints, than those from more predictable 125 environments (Canale and Henry 2010; Florant and Healy 2012; Geiser 2013). Therefore, 126 if food shortage is a major seasonal environmental constraint, we predict food 127 supplemented individuals to postpone their transition to winter phenotype, i.e. to 128 minimize torpor use relative to control individuals exposed to the seasonal, gradual reduction in food availability. 129

130

131 Materials and methods

132 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 140 141 (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 142 143 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 25 144 145 m intervals (Dammhahn and Kappeler 2008a). We compared the two plots in terms of 146 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 147 experiment, body condition of individuals from the control plot was 22% higher than in 148 149 individuals from the food supplemented plot. Unfortunately, we do not have the 150 necessary data to document the temporal covariation between body condition and torpor 151 use throughout the experiment. Because food supplementation is expected to induce a 152 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 153 154 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 155 because the sign, strength and significance of effects of the other variables remained 156 157 similar after the addition of a body condition effect (Table S3), we are confident that the 158 effect of food supplementation in our experimental design was not confounded by 159 ecological or physiological differences between the two plots.

160 Study animals were captured between 27-Mar and 08-Apr (1-Apr \pm 4 days), using 120 161 Sherman live traps baited with banana, opened at dusk and collected at dawn. Captured 162 individuals were kept in their trap for the day. Upon first capture, individuals were briefly 163 anesthetized (10 µl Ketanest 100), marked individually with subdermally implanted

microtransponders (Trovan, Germany), and measured (including body mass and head 164 165 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 166 167 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. 168 $T_{\rm sk}$ is a reliable indicator of core body temperature, at least for the characterization of 169 170 daily torpor use (Dausmann 2005). All captured individuals were released at their site of 171 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 ± 60 172 days) to retrieve the temperature loggers. Twenty-one individuals still equipped with 173 174 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 175 176 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. $T_{\rm sk}$ 177 data could be recovered from all loggers but one. Statistical analyses are based on 9 178 179 individuals from the control plot (5 females, 4 males) and 11 from the food supplemented 180 plot (4 females, 7 males), for which T_{sk} was recorded over 68 ± 14 days (range: 15 to 76 181 days; daily sample sizes per plot are presented in Table S4).

182

183 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

188 2008a). Food supplementation consisted of a mix of smashed banana, raisins and cat 189 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 190 ca. 270 kJ.day⁻¹ per feeder, which corresponds to daily energetic requirements of 3 to 4 191 individuals of 50 g body mass (Schmid and Ganzhorn 2009; Canale et al. 2011). Feeding 192 193 platform's design ascertained that they were highly attractive, and safe, for grey mouse 194 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 195 196 match their diet (Microgale and Geogale tenrecs, tufted-tailed rats Eliurus myoxinus), or 197 because they were inactive at that time of the year (fat-tailed dwarf lemurs, Cheirogaleus 198 *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 12-14-199 200 Jun; Table S4).

201

202 Characterization of torpor use

Continuous profiles of T_{sk} (see Tsk_rawdata; ESM) were used to compute three variables 203 204 that quantify the daily use of torpor (see Dataset; ESM). First, we determined whether an 205 individual entered torpor or not on a given day (i.e. individual probability to enter torpor: 206 P_{torp} , binomial variable; Table S4). Animals were considered torpid when their T_{sk} 207 dropped below 30°C (following Schmid 2001). When T_{sk} oscillated around 30°C (184 208 measures of 1357), we applied the rules presented in Fig. S3 to diagnose torpor bouts. 209 Second, for each identified torpor event, we computed the duration of torpor bout $(D_{torp},$ 210 in hours \pm 0.1), which corresponds to the time spent below 30°C during a day. These 211 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 212

to torpor by a torpid individual. Third, we also analysed minimal skin temperature (T_{sk} min, °C), which objectively quantifies hypothermia without relying on a threshold temperature. During torpor bouts, $T_{sk min}$ quantifies torpor depth (Vuarin et al. 2013).

216 Daily torpor use strongly depends on air temperature: the lower T_a during the resting 217 phase, the longer and the deeper torpor bouts (Heldmaier et al. 2004; Geiser 2013; Vuarin 218 et al. 2013). To adjust fluctuations in daily torpor use for temporal fluctuations in T_a , we 219 recorded T_a at resting sites in both plots. As grey mouse lemurs generally rest in tree 220 holes (Schmid 1998), we placed a temperature logger (iButtons, Maxim Integrated, USA) 221 in four tree holes per plot (as in Vuarin et al. 2013). T_a was recorded every 20 min 222 throughout the study period. We considered daily minimum T_a ($T_{a \min}$) in tree holes as a 223 reliable index of the thermal constraint faced by resting animals, since $T_{\rm sk\ min}$ is achieved during the coldest hours of the day (i.e., when T_a is the lowest; Schmid 2001; Vuarin et 224 225 al. 2013). Daily $T_{\rm a \ min}$ values for each plot were averaged across the four corresponding 226 temperature loggers. Thermal conditions were similar and highly correlated between the 227 two plots (Fig. 1; Table S1; Pearson's R = 0.92, p < 0.01).

228

229 Statistical analyses

As we recorded temporally repeated measures of P_{torp} , D_{torp} and T_{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} , $T_{sk min}$) 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}

min data collected after the start of the experiment, from 09-Apr to 23-Jun. P_{torp} data were 238 239 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 240 $T_{\rm sk\ min}$ data were analysed using linear mixed effects models using the 'lme' function of 241 242 'nlme' package (v. 3.1-109) for R. We adjusted torpor variables for the additive fixed 243 effects of time (assuming a linear effect of the number of days since the start of the 244 experiment with 09-Apr set to 1; temporal patterns other than linearity did not receive a better statistical support), sex and daily $T_{a \min}$, and their interactions (time and sex, time 245 and $T_{a \text{ min}}$, $T_{a \text{ min}}$ and sex). These variables influence daily torpor use: (1) low T_{a} is 246 247 associated with longer and deeper torpor bouts, (2) female grey mouse lemurs are less 248 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), 249 250 and (3) torpor is maximal during the height of the dry season (Schmid 2001).

251 To test our predictions, we added the fixed effect of experimental treatment (natural 252 food availability, named 'control' versus food supplementation, named 'food supplemented'), and its interactions with other fixed effects: (1) between treatment and 253 254 time to account for a potential change of the effect of treatment throughout the study 255 period, (2) between treatment and sex because females are more prone to use daily torpor, 256 and therefore might show a stronger response to the treatment than males, and (3) between treatment and $T_{a \min}$ as the effect of treatment could be countered by low T_{a} . 257 258 Finally, we also tested for an interaction (1) between treatment, time and sex effects 259 because the sex-dependence of the response to the treatment could change over time, and 260 (2) between time, sex and $T_{a \min}$ effects because the sex-dependence of the sensitivity to T_{a} 261 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 262

order 1 ('corAR1' function) for temporal dependence between residuals in the analyses of D_{torp} and $T_{sk min}$ (see Vuarin et al. 2013 for details). For the treatment effect, the control plot was defined as intercept.

266 Final models were obtained after removal of all non-significant interactions and 267 additive effects based on likelihood ratio tests between nested models. We proceeded in 268 three steps: assessment of third order interactions, then of second order interactions and, 269 eventually, of additive effects in presence of significant, non-nested interactions. At each 270 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 271 272 checked whether residuals of each final model followed a Gaussian distribution. Slopes 273 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, 274 275 stepwise model selection. Torpor parameter estimates are presented as mean \pm standard deviation (SD). 276

277

278 **Results**

Over the nine days before the start of the experiment, torpor parameters did not differ 279 between the two plots (respectively for P_{torp} , and $T_{\text{sk min}}$: $\chi^{2}_{1} = 0.74$, p = 0.39, $\beta = 1.75 \pm$ 280 2.55 and $\chi^2_1 = 0.78$, p = 0.38, $\beta = -0.50 \pm 0.62^{\circ}$ C). The percentage of individuals entering 281 torpor on a given day (P_{torp}) was 12.5% in the control plot and 3.2% in the food 282 supplemented one (respectively, 3 of 9, and 1 of 7 individuals entered torpor at least 283 once). $T_{\rm sk\ min}$ was 30.6 ± 1.6°C in the control plot and 31.1 ± 1.0°C in the food 284 285 supplemented one. D_{torp} was 1.5 ± 1.3 h in the control plot and 0.5 h in the food 286 supplemented plot for the single individual that used torpor once.

287 After the start of food supplementation, torpor use rapidly differed between the two 288 plots (Fig. 2). Ptorp was much lower in the food-supplemented plot than in the control one 289 (Table 1). On average throughout the experiment, control individuals expressed torpor on 290 93.4% of the days sampled (N = 672 individual days), whereas food-supplemented 291 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 $T_{a \min}$, with a general trend for 292 293 increasing torpor use throughout the experiment, which was reinforced by low $T_{a \min}$ (time x $T_{a \min}$ interaction; Table 1), and for time and sex, with males increasing their use of 294 295 torpor later than females (time x sex interaction; Table 1) (Fig. 2). However, the response 296 to the food treatment did not vary through time (treatment x time interaction, Table 1), 297 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 $T_{a \min}$ interaction, Table 1). 298

299 Among individuals using torpor, food supplementation reduced D_{torp} : control individuals spent more time torpid (12.7 \pm 7.2 h) than food supplemented ones (3.3 \pm 6.4 300 301 h; Table 1; Fig. 3A). Torpor duration increased through time and increased with decreasing $T_{a \min}$ (Table 1), regardless the food treatment (treatment x time and treatment 302 303 x $T_{a \min}$ interactions; Table 1). This sensitivity of torpor duration to $T_{a \min}$ decreased 304 throughout the season (time x $T_{a \min}$ interaction, Table 1). Males exhibited shorter torpor bouts than females $(1.8 \pm 5.0 \text{ versus } 6.3 \pm 7.7 \text{ h}$ for the food supplemented plot and 11.6 305 \pm 7.3 versus 13.6 \pm 7.1 h for the control plot; Table 1), regardless the food treatment 306 307 (treatment x sex interaction; Table 1).

308 Grey mouse lemurs from the food-supplemented plot maintained higher $T_{sk min}$ during 309 rest than those from the control plot (Table 1). Control individuals exhibited an average 310 $T_{sk min}$ of 20.7 ± 4.9°C whereas $T_{sk min}$ of supplemented ones was 26.7 ± 5.8°C. This was 311 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 $T_{\rm sk\ min}$ than control males (treatment x time x sex interaction; Table 1; Fig. 3B). There was a general trend for decreasing $T_{\rm sk\ min}$ with time (i.e. increasing torpor depth; Table 1). Furthermore, $T_{\rm sk\ min}$ decreased with decreasing $T_{\rm a\ min}$ (Table 1), and this sensitivity of $T_{\rm sk}$ min to $T_{\rm a\ min}$ increased throughout the season (time x $T_{\rm a\ min}$ interaction, Table 1). Finally, males were less responsive to $T_{\rm a\ min}$ than females ($T_{\rm a\ min}$ x sex interaction, Table 1).

318

319 **Discussion**

320 This food supplementation experiment demonstrated that the increasing use of torpor at 321 the transition between the wet season and the dry season is flexible, and adjusted to actual 322 food availability in a free-ranging heterotherm. Individuals with access to supplementary 323 food reduced torpor use, duration and depth. These results demonstrate that the timing of 324 winter phenotype expression is fine-tuned according to energy availability, superseding 325 the influence of abiotic cues of seasonal transition, such as photoperiod and climatic 326 conditions. Nonetheless, daily torpor flexibility proved to be limited to the first months of 327 the transition to the dry season. One to two months later than control individuals, food 328 supplemented ones also switched to a winter phenotype, with regular daily torpor, despite 329 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 337 338 (hoard of seeds) and on prolonged bouts of hypometabolism to survive the 4-7 months 339 cold, boreal winter. Individuals with experimentally enlarged food stores spent more time 340 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 341 342 regulation in free-ranging animals, despite major differences in ecological and energetic 343 constraints operating in the different systems (Vuarin and Henry 2014). They either highlighted correlative evidence of the dependence of heterothermy use on food 344 availability under natural conditions, experimentally manipulated food availability under 345 346 semi-natural conditions, or indirectly induced food shortage through an experimental 347 increase of intraspecific competition for food. One compelling correlative study showed that the probability to use torpor in Australian owlet-nightjars (Aegotheles cristatus) 348 349 depended primarily on insect availability, and to a lesser extent on rainfall, with no 350 influence of low air temperature (Doucette et al. 2012).

351 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 352 phenology supported the assumption that fleshy fruit availability and flying insect 353 354 abundance decrease to a minimal level during the heart of the dry season (Dammhahn 355 and Kappeler 2008b). During our experiment, meteorological conditions corresponded to a typical start of the dry season (Schmid and Kappeler 1998; Dammhahn and Kappeler 356 357 2008b), with an abrupt stop of rainfall and decreasing night temperatures (Fig. 1), 358 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 359 360 heterothermic responses of control individuals demonstrate that our experiment actually took place at the ecological transition between wet and dry seasons. 361

362 If food availability was the only determinant of torpor use, food supplemented 363 individuals should have maintained their summer phenotype (i.e., normothermia) throughout the experiment. However, they started to regularly enter torpor after one (for 364 365 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 366 exclusive hypotheses could explain these temporal dynamics of the effect of food 367 368 supplementation on torpor use. First, the influence of anticipatory seasonal cues (photoperiod) on torpor regulation may increase through time, as the predictable risk of 369 resource shortage increases. At the onset of the dry season, daily torpor would be flexibly 370 371 adjusted to proximal environmental cues reflecting actual ecological conditions. Later on, 372 the influence of photoperiod would take over that of proximal cues of resource constraints to guarantee that individuals enter into the adaptive physiological and 373 374 behavioural states that maximize survival chances to the predictable, harshest part of the 375 dry season. The existence of such a time window of reduced influence of photoperiod 376 relative to proximal cues during the transition between the favourable and the 377 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. 378 379 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 380 (Lambrechts and Perret 2000; Dawson 2008). Moreover, Paul et al. (2009) 381 382 experimentally demonstrated in captive Siberian hamsters (Phodopus sungorus) that non-383 photic cues (food availability, social structure) inhibit the reproductive axis under autumn 384 photoperiod, but not under summer photoperiod. Overall, these observations suggest that, 385 during seasonal transitions, phenotypic change is facultative and largely influenced by 386 proximal cues of environmental constraints, whereas it becomes obligate and mainly

387 driven by photoperiod when the animals enters the height of the harsh season (winter/dry 388 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 389 390 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, 391 392 than lean individuals (Vuarin et al. 2013). Grey mouse lemurs would have used 393 supplementary food to constitute larger fat reserves than usual, postponing their physiological shift to winter phenotype. According to the asset protection principle 394 395 (Dammhahn 2012), food-supplemented animals would have initiated regular daily torpor 396 use when the prospect of supplementary fat storage was offset by the costs of additional 397 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 398 399 efficiency of conversion of supplemented food into fat stores (Sheriff et al. 2013a; Giroud 400 et al. 2014). Third, natural food availability (Dammhahn and Kappeler 2008a) is expected 401 to decrease through time. Grey mouse lemurs may have remained normothermic as long 402 as natural food availability, supplemented by artificial food provisioning, was sufficient to fulfil their daily energetic requirements. But when natural food supplies were 403 404 exhausted, individuals would have started to use daily torpor to counterbalance growing 405 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 412 413 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 414 415 temperatures throughout the experiment than supplemented females, who ended up exhibiting similar patterns than control individuals (Fig. 3). These sex-specific 416 417 differences in seasonal timing of daily torpor use might result from reduced body 418 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. 419 420 2013), or from sex-differences in energetic requirements to engage in reproduction just 421 after the dry season (Schmid and Kappeler 1998; Canale et al. 2012; see also Munro et al. 422 2005; Sheriff et al. 2013b). Our study is the first to document, in free-ranging grey mouse 423 lemurs, that sex differences in activity patterns (Rasoazanabary 2006) result from the fact 424 that males and females do not start using daily torpor at the same period of the dry 425 season, males entering daily torpor later than females.

To conclude, the transition to winter phenotype is plastic and not primarily timed 426 according to abiotic cues that allow the anticipation of the dry season. During the 427 transition between wet and dry seasons, the seasonal transition to winter phenotype is 428 429 fine-tuned according to food availability. The cues used to time the final, food-430 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 431 432 our changing world, identification of the cues that time heterothermy is an obligate 433 preliminary step to determine which, and how, heterothermic animals could be resilient to 434 human-induced disturbances of environmental cycles, like habitat destruction or climate 435 change (Canale and Henry 2010; Lane et al. 2012; Geiser 2013; Williams et al. 2014).

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449 **Bibliography**

- Angilletta MJ, Cooper BS, Schuler MS, Boyles JG (2010) The evolution of thermal
 physiology in endotherms. Front Biosci E2:861–881
- Auld JR, Agrawal AA, Relyea RA (2010) Re-evaluating the costs and limits of adaptive
 phenotypic plasticity. Proc R Soc B 277:503–511
- Bieber C, Juškaitis R, Turbill C, Ruf T (2012) High survival during hibernation affects
 onset and timing of reproduction. Oecologia 169:155–166
- Bieber C, Lebl K, Stalder G, Geiser F, Ruf T (2014) Body mass dependent use of
 hibernation: why not prolong the active season, if they can? Funct Ecol 28:167–177
- 458 Canale CI, Henry P-Y (2010) Adaptive phenotypic plasticity and resilience of vertebrates
 459 to increasing climatic unpredictability. Clim Res 43:135–147
- Canale CI, Perret M, Henry P-Y (2012) Torpor use during gestation and lactation in a
 primate. Naturwissenschaften 99:159–163
- 462 Canale CI, Perret M, Théry M, Henry P-Y (2011) Physiological flexibility and
 463 acclimation to food shortage in a heterothermic primate. J Exp Biol 214:551–560

- 464 Cornelius JM, Boswell T, Jenni-Eiermann S, Breuner CW, Ramenofsky M (2013)
 465 Contributions of endocrinology to the migration life history of birds. Gen Comp
 466 Endocrinol 190:47–60
- 467 Dammhahn M (2012) Are personality differences in a small iteroparous mammal
 468 maintained by a life-history trade-off? Proc R Soc B 279:2645–2651
- Dammhahn M, Kappeler PM (2008a) Small-scale coexistence of two mouse lemur
 species (*Microcebus berthae* and *M. murinus*) within a homogeneous competitive
 environment. Oecologia 157:473–483
- 472 Dammhahn M, Kappeler PM (2008b) Comparative feeding ecology of sympatric
 473 *Microcebus berthae* and *M. murinus*. Int J Primatol 29:1567–1589
- Dammhahn M, Kappeler PM (2013) Seasonality and behavioral energy strategies in
 Microcebus berthae and *M. murinus*. In: Masters J, Gamba M, Génin F (eds)
 Leaping Ahead Advances in Prosimian Biology. Springer, New York, pp 215–223
- 477 Dausmann KH (2005) Measuring body temperature in the field-evaluation of external vs.
 478 implanted transmitters in a small mammal. J Therm Biol 30:195–202
- 479 Dawson A (2008) Control of the annual cycle in birds: endocrine constraints and
 480 plasticity in response to ecological variability. Philos Trans R Soc B 363:1621–1633
- 481 Dewar RE, Richard AF (2007) Evolution in the hypervariable environment of
 482 Madagascar. Proc Natl Acad Sci 104:13723–13727
- 483 Doucette LI, Brigham RM, Pavey CR, Geiser F (2012) Prey availability affects daily
 484 torpor by free-ranging Australian owlet-nightjars (*Aegotheles cristatus*). Oecologia
 485 169:361–372
- Florant GL, Healy JE (2012) The regulation of food intake in mammalian hibernators: a
 review. J Comp Physiol B 182:451–467
- 488 Geiser F (2013) Hibernation. Curr Biol 23:R188–R193
- 489 Geiser F (2004) Metabolic rate and body temperature reduction during hibernation and
 490 daily torpor. Annu Rev Physiol 66:239–274
- Génin F, Perret M (2003) Daily hypothermia in captive grey mouse lemurs (*Microcebus murinus*): effects of photoperiod and food restriction. Comp Biochem Physiol Part B 136:71–81
- 494 Gienapp P, Leimu R, Merilä J (2007) Responses to climate change in avian migration
 495 time-microevolution versus phenotypic plasticity. Clim Res 35:25–35
- Giroud S, Blanc S, Aujard F, Bertrand F, Gilbert C, Perret M (2008) Chronic food
 shortage and seasonal modulations of daily torpor and locomotor activity in the grey

- 498 mouse lemur (*Microcebus murinus*). Am J Physiol Regul Integr Comp Physiol
 499 294:R1958–R1967
- Giroud S, Perret M, Gilbert C, Zahariev A, Goudable J, Le Maho Y, Oudart H, Momken
 I, Aujard F, Blanc S (2009) Dietary palmitate and linoleate oxidations, oxidative
 stress, and DNA damage differ according to season in mouse lemurs exposed to a
 chronic food deprivation. Am J Physiol Regul Integr Comp Physiol 297:R950–
 R959
- 505
- Giroud S, Zahn S, Criscuolo F, Chery I, Blanc S, Turbill C, Ruf T (2014) Late-born
 intermittently fasted juvenile garden dormice use torpor to grow and fatten prior to
 hibernation: consequences for ageing processes. Proc R Soc B 281: 20143111
- Heldmaier G, Ortmann S, Elvert R (2004) Natural hypometabolism during hibernation
 and daily torpor in mammals. Respir Physiol Neurobiol 141:317–329
- Helm B, Ben-Shlomo R, Sheriff MJ, et al. (2013) Annual rhythms that underlie
 phenology: biological time-keeping meets environmental change. Proc R Soc B
 280:20130016
- Humphries MM, Thomas DW, Kramer DL (2003a) The role of energy availability in
 mammalian hibernation: a cost-benefit approach. Physiol Biochem Zool 76:165–179
- Humphries MM, Kramer DL, Thomas DW (2003b) The role of energy availability in
 mammalian hibernation: an experimental test in free-ranging eastern chipmunks.
 Physiol Biochem Zool 76:180–186
- Kobbe S, Ganzhorn JU, Dausmann KH (2011) Extreme individual flexibility of
 heterothermy in free-ranging Malagasy mouse lemurs (*Microcebus griseorufus*). J
 Comp Physiol B 181:165–173
- Körtner G, Geiser F (2000) The temporal organization of daily torpor and hibernation:
 circadian and circannual rhythms. Chronobiol Int 17:103–128
- Kraus C, Eberle M, Kappeler PM (2008) The costs of risky male behaviour: sex
 differences in seasonal survival in a small sexually monomorphic primate. Proc R
 Soc B 275:1635–1644
- Lambrechts MM, Perret P (2000) A long photoperiod overrides non-photoperiodic factors
 in blue tits' timing of reproduction. Proc R Soc B 267:585–588
- Landry-Cuerrier M, Munro D, Thomas DW, Humphries MM (2008) Climate and
 resource determinants of fundamental and realized metabolic niches of hibernating
 chipmunks. Ecology 89:3306–3316
- Lane JE, Kruuk LEB, Charmantier A, Murie JO, Dobson FS (2012) Delayed phenology
 and reduced fitness associated with climate change in a wild hibernator. Nature
 489:554–557

- Miller-Rushing AJ, Høye TT, Inouye DW, Post E (2010) The effects of phenological
 mismatches on demography. Philos Trans R Soc B 365:3177–3186
- Munro D, Thomas DW, Humphries MM (2005) Torpor patterns of hibernating eastern
 chipmunk *Tamias striatus* vary in response to the size and fatty acid composition of
 food hoards. J Anim Ecol 74:692–700
- Ozgul A, Childs DZ, Oli MK, Armitage KB, Blumstein DT, Olson LE, Tuljapurkar S,
 Coulson T (2010) Coupled dynamics of body mass and population growth in
 response to environmental change. Nature 466:482–485
- Patil VP, Morrison SF, Karels TJ, Hik DS (2013) Winter weather versus group
 thermoregulation: what determines survival in hibernating mammals? Oecologia
 173:139–149
- Paul MJ, Galang J, Schwartz WJ, Prendergast BJ (2009) Intermediate-duration day
 lengths unmask reproductive responses to nonphotic environmental cues. Am J
 Physiol Regul Integr Comp Physiol 296:R1613–R1619
- Perret M, Aujard F (2001) Regulation by photoperiod of seasonal changes in body mass
 and reproductive function in gray mouse lemurs (*Microcebus murinus*): differential
 responses by sex. Int J Primatol 22:5–24
- Piersma T, Drent J (2003) Phenotypic flexibility and the evolution of organismal design.
 Trends Ecol Evol 18:228–233
- Rasoazanabary E (2006) Male and female activity patterns in *Microcebus murinus* during
 the dry season at Kirindy forest, western Madagascar. Int J Primatol 27:437–464
- Reed TE, Waples RS, Schindler DE, Hard JJ, Kinnison MT (2010) Phenotypic plasticity
 and population viability: the importance of environmental predictability. Proc R
 Soc B 277:3391–400
- Schmid J (1998) Tree holes used for resting by gray mouse lemurs (*Microcebus murinus*)
 in Madagascar: insulation capacities and energetic consequences. Int J Primatol
 19:797–809
- Schmid J (2001) Daily torpor in free-ranging gray mouse lemurs (*Microcebus murinus*)
 in Madagascar. Int J Primatol 22:1021–1031
- Schmid J, Ganzhorn JU (2009) Optional strategies for reduced metabolism in gray mouse
 lemurs. Naturwissenschaften 96:737–741
- Schmid J, Kappeler PM (1998) Fluctuating sexual dimorphism and differential
 hibernation by sex in a primate, the gray mouse lemur (*Microcebus murinus*). Behav
 Ecol Sociobiol 43:125–132

- Sheriff MJ, Kenagy GJ, Richter M, Lee T, Tøien O, Kohl F, Buck CL, Barnes BM (2011)
 Phenological variation in annual timing of hibernation and breeding in nearby
 populations of arctic ground squirrels. Proc R Soc B 278:2369–2375
- Sheriff MJ, Williams CT, Kenagy GJ, Buck CL, Barnes BM (2012) Thermoregulatory
 changes anticipate hibernation onset by 45 days: data from free-living arctic ground
 squirrels. J Comp Physiol B 182:841–847
- Sheriff MJ, Fridinger RW, Tøien Ø, Barnes BM, Buck CL (2013a) Metabolic rate and
 prehibernation fattening in free-living arctic ground squirrels. Physiol Biochem Zool
 86:515–27
- Sheriff MJ, Richter MM, Buck CL, Barnes BM (2013b) Changing seasonality and
 phenological responses of free-living male arctic ground squirrels: the importance of
 sex. Philos Trans R Soc B 368:20120480
- 581 Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford
- Terrien J, Perret M, Aujard F (2010) Gender markedly modulates behavioral
 thermoregulation in a non-human primate species, the mouse lemur (*Microcebus murinus*). Physiol Behav 101:469–473
- Thomas DW, Blondel J, Perret P, Lambrechts MM, Speakman JR (2001) Energetic and
 fitness costs of mismatching resource supply and demand in seasonally breeding
 birds. Science 291:2598–2600
- Turbill C, Bieber C, Ruf T (2011) Hibernation is associated with increased survival and
 the evolution of slow life histories among mammals. Proc R Soc B 278:3355–3363
- Visser ME, Caro SP, van Oers K, Schaper SV, Helm B (2010) Phenology, seasonal
 timing and circannual rhythms: towards a unified framework. Philos Trans R Soc B
 365:3113–3127
- Vuarin P, Dammhahn M, Henry P-Y (2013) Individual flexibility in energy saving: body
 size and condition constrain torpor use. Funct Ecol 27:793–799
- Vuarin P, Henry P-Y (2014) Field evidence for a proximate role of food shortage in the
 regulation of hibernation and daily torpor: a review. J Comp Physiol B 184:683–697
- Williams CT, Barnes BM, Kenagy GJ, Buck CL (2014) Phenology of hibernation and
 reproduction in ground squirrels: integration of environmental cues with endogenous
 programming. J Zool 292:112–124

Table 1 Effects of treatment, minimal air temperature ($T_{a \min}$), 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 ($T_{sk \min}$), for 20 free-ranging grey mouse lemurs (*Microcebus murinus*).

	P torp									<i>D</i> _{torp}								T _{sk min}							
	Full model				Final model				Full model				Final model				Full model				Final model				
	β	SE	Z value	Р	β	SE	χ²	р	β	SE	t-value	Р	β	SE	χ²	р	β	SE	t-value	Р	β	SE	χ²	р	
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 _{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 _{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 _{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 _{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 _{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 (\pm 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 (\Im) and female (\Im) 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 (\pm SD; dots) and predicted (lines) torpor bout duration for (a, b) control and (c, d) food supplemented male (\Im) and female (\Im) grey mouse lemurs according to the date (each dot corresponds to the mean \pm SD of the duration for the day \pm 2 days); and observed (\pm SD; dots) and predicted (lines) minimal skin temperature for (e, f) control and (g, h) food supplemented male (\Im) and female (\Im) grey mouse lemurs according to the date (each dot corresponds to the mean \pm SD of the temperature for the day \pm 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 2





