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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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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  
31 mouse lemurs, *Microcebus murinus*), at the transition to the food-limited dry season, i.e.  
32 the austral winter. As expected under the food shortage hypothesis, food supplemented  
33 individuals postponed the seasonal transition to normal torpor use by 1-2 month(s), spent  
34 4 time less torpid, and exhibited minimal skin temperature 6°C higher than control  
35 animals. This study provides the first *in situ* experimental evidence that food availability,  
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  
39 fluctuations.

40

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

43

## 44 **Introduction**

45 Understanding how individuals allocate energy between competing life history traits  
46 (Stearns 1992) and under changing environmental conditions (Visser et al. 2010; Lane et  
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

68 heterothermy as the faculty of some endotherms to undergo a controlled reduction of their  
69 body temperature during bouts of hibernation and daily torpor, in association with  
70 metabolism reduction (Heldmaier et al. 2004; Geiser 2013). Timing of switches to winter  
71 phenotype should be crucial for the chances of free-ranging heterotherms to survive and  
72 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  
75 commonly been regarded as an adaptive strategy to cope with energy shortage  
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  
83 (Visser et al. 2010). A major risk when compensating environmental changes by  
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  
94 seasonal acclimation (Helm et al. 2013). But proximate energetic constraints also  
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  
104 direct experimental approach to test for the dependence of heterothermy on energy  
105 availability (Humphries et al. 2003b; Vuarin and Henry 2014). The aim of the present  
106 study was to provide the first test of a direct dependence of the launching of torpor use on  
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

116 to maintain a favourable energy balance. Grey mouse lemurs enter torpor mostly on a  
117 daily basis (Schmid 2001; Fig. S3) with large inter-individual variation in its expression  
118 (Vuarin et al. 2013). Because they evolved in a highly seasonal environment, but with  
119 poorly predictable intra- and inter-annual fluctuations in resource availability (Dewar and  
120 Richard 2007), we predict that they directly cue in on local resource availability to time  
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  
123 unpredictable environments are indeed expected to be more phenotypically flexible, and  
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  
129 reduction in food availability.

130

## 131 **Materials and methods**

### 132 Study site and animals

133 The study was conducted in the dry deciduous forest of Kirindy/CNFEREF (44°39'E,  
134 20°03'S, 30-60 m above sea level) in western Madagascar between March and October  
135 2010. The climate is highly seasonal, with a hot wet season from December to March and  
136 a cool dry season with little or no rain from April to November (Dammhahn and  
137 Kappeler 2008a). Throughout the experiment from April to June, mean  $T_a$  was  $23.9 \pm$   
138  $7.4^\circ\text{C}$ , and it rained only twice, before the start of the experiment (13.7 mm on 12-Mar  
139 and 2.2 mm on 13-Mar).

140 Grey mouse lemurs forage in overlapping home ranges and do not cache food  
141 (Dammhahn and Kappeler 2008b). Hence, our experimental design had to be based on  
142 the comparison of daily torpor patterns between a control plot (named N5) and a plot with  
143 food supplementation (named CS5). The two plots are ca. 2 km apart within a continuous  
144 forest and defined by boundaries of grid systems (500 x 500 m) of small foot trails at 25  
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  
147 and torpor use (ESM1, Table S1). The only notable difference was that, before the  
148 experiment, body condition of individuals from the control plot was 22% higher than in  
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  
153 predictor of body condition days and weeks later during the experiment. We still assessed  
154 the robustness of our results to initial differences in body condition between plots  
155 (ESM2). Because effects related to body condition were non-significant (Table S2), and  
156 because the sign, strength and significance of effects of the other variables remained  
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  $\mu$ l Ketanest 100), marked individually with subdermally implanted



164 microtransponders (Trovan, Germany), and measured (including body mass and head  
165 width; Dammhahn and Kappeler 2008a; Vuarin et al. 2013). Forty-three adult (i.e. > 50  
166 g) individuals, 19 from the control plot and 24 from the food supplemented plot, were  
167 equipped with collar-mounted temperature loggers (~3 g, Weetag Lite, AlphaMach,  
168 Sainte-Julie, Canada; Fig. S1), which recorded skin temperature ( $T_{sk}$ ) every 10 minutes.  
169  $T_{sk}$  is a reliable indicator of core body temperature, at least for the characterization of  
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  
172 experiment, study animals were recaptured between 09-Jun and 03-Nov (12-Aug  $\pm$  60  
173 days) to retrieve the temperature loggers. Twenty-one individuals still equipped with  
174 loggers were recaptured (8 loggers out of 21 were retrieved  $13 \pm 4$  days (range: 9–19-  
175 Jun) before the end of the food supplementation to maximize recovery rates as some  
176 individuals had already lost their collars at the beginning of June). Twelve other  
177 individuals were recaptured but had lost their collars, and 10 individuals disappeared.  $T_{sk}$   
178 data could be recovered from all loggers but one. Statistical analyses are based on 9  
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  $68 \pm 14$  days (range: 15 to 76  
181 days; daily sample sizes per plot are presented in Table S4).

182

### 183 Food supplementation design

184 Food supplementation started on 08-Apr and lasted until 23-Jun (77 days) (Table S4). In  
185 the food supplemented plot, food was provided daily at 12 feeding platforms (Fig. S2)  
186 that were distributed across the plot according to locations of mouse lemurs aggregates.  
187 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.  
190 144 kJ per feeder) provided on the following day. Mean daily energetic provisioning was  
191 ca. 270 kJ.day<sup>-1</sup> per feeder, which corresponds to daily energetic requirements of 3 to 4  
192 individuals of 50 g body mass (Schmid and Ganzhorn 2009; Canale et al. 2011). Feeding  
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  
195 were unlikely to feed at the platforms either because the type of food provided did not  
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  
199 nights every second week (17–19Apr, 01–03-May, 15–17-May, 29–31-May and 12–14-  
200 Jun; Table S4).

201

## 202 Characterization of torpor use

203 Continuous profiles of  $T_{sk}$  (see Tsk\_rawdata; ESM) were used to compute three variables  
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  
212 supplementation on the probability to enter torpor ( $P_{torp}$ ) and the amount of time allocated

213 to torpor by a torpid individual. Third, we also analysed minimal skin temperature ( $T_{sk}$   
214  $_{min}$ , °C), which objectively quantifies hypothermia without relying on a threshold  
215 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  $T_{sk\ min}$  is achieved  
224 during the coldest hours of the day (i.e., when  $T_a$  is the lowest; Schmid 2001; Vuarin et  
225 al. 2013). Daily  $T_{a\ min}$  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

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

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

238  $T_{a \min}$  data collected after the start of the experiment, from 09-Apr to 23-Jun.  $P_{\text{torp}}$  data were  
239 analysed with generalized linear mixed effects models fitted with a binomial distribution,  
240 using the ‘lmer’ function of ‘lme4’ package (v. 0.999999-2) for R, whereas both  $D_{\text{torp}}$  and  
241  $T_{\text{sk min}}$  data were analysed using linear mixed effects models using the ‘lme’ function of  
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  
245 better statistical support), sex and daily  $T_{a \min}$ , and their interactions (time and sex, time  
246 and  $T_{a \min}$ ,  $T_{a \min}$  and sex). These variables influence daily torpor use: (1) low  $T_a$  is  
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  
249 Kappeler 1998; Kraus et al. 2008; Dammhahn and Kappeler 2008b; Terrien et al. 2010),  
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  
253 supplemented’), and its interactions with other fixed effects: (1) between treatment and  
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)  
257 between treatment and  $T_{a \min}$  as the effect of treatment could be countered by low  $T_a$ .  
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  
262 due to temporal autocorrelation, we also parameterized an auto-regressive structure of

263 order 1 ('corAR1' function) for temporal dependence between residuals in the analyses of  
264  $D_{\text{torp}}$  and  $T_{\text{sk min}}$  (see Vuarin et al. 2013 for details). For the treatment effect, the control  
265 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  
271 with a model adjusted for all others. The significance level was set to  $p < 0.05$ . We  
272 checked whether residuals of each final model followed a Gaussian distribution. Slopes  
273 of effects ( $\beta$ ), and their standard errors (SE), are presented for full models and final  
274 models to allow assessment of the effects to the potential weaknesses of downward,  
275 stepwise model selection. Torpor parameter estimates are presented as mean  $\pm$  standard  
276 deviation (SD).

277

## 278 **Results**

279 Over the nine days before the start of the experiment, torpor parameters did not differ  
280 between the two plots (respectively for  $P_{\text{torp}}$ , and  $T_{\text{sk min}}$ :  $\chi^2_1 = 0.74$ ,  $p = 0.39$ ,  $\beta = 1.75 \pm$   
281  $2.55$  and  $\chi^2_1 = 0.78$ ,  $p = 0.38$ ,  $\beta = -0.50 \pm 0.62^\circ\text{C}$ ). The percentage of individuals entering  
282 torpor on a given day ( $P_{\text{torp}}$ ) was 12.5% in the control plot and 3.2% in the food  
283 supplemented one (respectively, 3 of 9, and 1 of 7 individuals entered torpor at least  
284 once).  $T_{\text{sk min}}$  was  $30.6 \pm 1.6^\circ\text{C}$  in the control plot and  $31.1 \pm 1.0^\circ\text{C}$  in the food  
285 supplemented one.  $D_{\text{torp}}$  was  $1.5 \pm 1.3$  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).  $P_{\text{torp}}$  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  
292 days). These results are adjusted for the effects of time and  $T_{a \text{ min}}$ , with a general trend for  
293 increasing torpor use throughout the experiment, which was reinforced by low  $T_{a \text{ min}}$  (time  
294 x  $T_{a \text{ min}}$  interaction; Table 1), and for time and sex, with males increasing their use of  
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  
298 was not reinforced by low  $T_a$  either (treatment x  $T_{a \text{ min}}$  interaction, Table 1).

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

308 Grey mouse lemurs from the food-supplemented plot maintained higher  $T_{\text{sk min}}$  during  
309 rest than those from the control plot (Table 1). Control individuals exhibited an average  
310  $T_{\text{sk min}}$  of  $20.7 \pm 4.9^\circ\text{C}$  whereas  $T_{\text{sk min}}$  of supplemented ones was  $26.7 \pm 5.8^\circ\text{C}$ . This was  
311 no longer the case at the end of the experiment when food-supplemented females reached

312 values similar to control females, while food-supplemented males still exhibited higher  
313  $T_{sk\ min}$  than control males (treatment x time x sex interaction; Table 1; Fig. 3B). There  
314 was a general trend for decreasing  $T_{sk\ min}$  with time (i.e. increasing torpor depth; Table 1).  
315 Furthermore,  $T_{sk\ min}$  decreased with decreasing  $T_{a\ min}$  (Table 1), and this sensitivity of  $T_{sk}$   
316  $_{min}$  to  $T_{a\ min}$  increased throughout the season (time x  $T_{a\ min}$  interaction, Table 1). Finally,  
317 males were less responsive to  $T_{a\ min}$  than females ( $T_{a\ min}$  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.

330 As predicted, grey mouse lemurs flexibly adjusted their use of torpor according to  
331 food availability, supporting the torpor optimization hypothesis (Humphries et al. 2003a),  
332 i.e. individuals use available energy to minimize heterothermy expression, which in turn  
333 reduces associated costs (Humphries et al. 2003a; Angilletta et al. 2010). The two other  
334 experiments of food supplementation with free-ranging heterotherms also support a  
335 proximate role of food availability in heterothermy regulation (Humphries et al. 2003b;  
336 Munro et al. 2005). They were both conducted with a temperate hibernator, the eastern

337 chipmunk (*Tamias striatus*) during winter. Chipmunks rely on external energy storage  
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.  
341 2005). Six other studies support a proximate role of food availability in heterothermy  
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  
344 highlighted correlative evidence of the dependence of heterothermy use on food  
345 availability under natural conditions, experimentally manipulated food availability under  
346 semi-natural conditions, or indirectly induced food shortage through an experimental  
347 increase of intraspecific competition for food. One compelling correlative study showed  
348 that the probability to use torpor in Australian owlet-nightjars (*Aegotheles cristatus*)  
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  
352 decreases during the transition to the dry season. Monitoring of insect biomass and plant  
353 phenology supported the assumption that fleshy fruit availability and flying insect  
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  
356 a typical start of the dry season (Schmid and Kappeler 1998; Dammhahn and Kappeler  
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  
359 the control plot (Fig. 2; e.g., Schmid 2001). Overall, meteorological conditions and  
360 heterothermic responses of control individuals demonstrate that our experiment actually  
361 took place at the ecological transition between wet and dry seasons.



362 If food availability was the only determinant of torpor use, food supplemented  
363 individuals should have maintained their summer phenotype (i.e., normothermia)  
364 throughout the experiment. However, they started to regularly enter torpor after one (for  
365 females) to two (for males) months of food supplementation, and most individuals  
366 entered torpor by the end of the experiment (end of June; Fig. 2). Three non-mutually  
367 exclusive hypotheses could explain these temporal dynamics of the effect of food  
368 supplementation on torpor use. First, the influence of anticipatory seasonal cues  
369 (photoperiod) on torpor regulation may increase through time, as the predictable risk of  
370 resource shortage increases. At the onset of the dry season, daily torpor would be flexibly  
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  
373 constraints to guarantee that individuals enter into the adaptive physiological and  
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  
378 constraints, has already been suggested in other heterotherms (Geiser 2004; Munro et al.  
379 2005; Sheriff et al. 2012). It also allows birds to fine-tune the timing of spring migration  
380 (Cornelius et al. 2013) and reproduction initiation according to local conditions  
381 (Lambrechts and Perret 2000; Dawson 2008). Moreover, Paul et al. (2009)  
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,  
389 rather than directly by food availability. Anticipatory fattening is a prerequisite for  
390 sustained regular daily torpor and hibernation (Körtner and Geiser 2000). And individuals  
391 with high body condition are more prone to use daily torpor, and use it more flexibly,  
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  
394 physiological shift to winter phenotype. According to the asset protection principle  
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.  
398 2011; Bieber et al. 2014). The first bouts of daily torpor may have even increased the  
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  
403 to fulfil their daily energetic requirements. But when natural food supplies were  
404 exhausted, individuals would have started to use daily torpor to counterbalance growing  
405 energetic deficits associated with insufficient supply of artificial food.

406 In grey mouse lemurs, as in other heterotherms (like eastern chipmunks or arctic  
407 ground squirrels *Urocitellus parryii*; Munro et al. 2005; Sheriff et al. 2011, 2013b), males  
408 use less daily torpor than females. They remain more active and can be captured  
409 throughout the dry season (Schmid and Kappeler 1998; Kraus et al. 2008; Dammhahn  
410 and Kappeler 2008b; Dammhahn and Kappeler 2013). Our results clarify this sex  
411 difference in daily torpor use: at the heart of the dry season (June), males expressed daily

412 torpor as much as females in the control plot, although less than females in the food  
413 supplemented plot (Figs 2-3). The main difference is that males begun regular daily  
414 torpor use one month later than females (Fig. 2), and exhibited higher minimal skin  
415 temperatures throughout the experiment than supplemented females, who ended up  
416 exhibiting similar patterns than control individuals (Fig. 3). These sex-specific  
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),  
419 and associated reduced propensity to use daily torpor (Kobbe et al. 2011; Vuarin et al.  
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.

426 To conclude, the transition to winter phenotype is plastic and not primarily timed  
427 according to abiotic cues that allow the anticipation of the dry season. During the  
428 transition between wet and dry seasons, the seasonal transition to winter phenotype is  
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  
431 photoperiod, which would override the influence of current environmental conditions. In  
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).

436

437

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

	$P_{\text{torp}}$								$D_{\text{torp}}$								$T_{\text{sk min}}$							
	Full model				Final model				Full model				Final model				Full model				Final model			
	$\beta$	SE	Z value	$P$	$\beta$	SE	$\chi^2$	$p$	$\beta$	SE	t-value	$P$	$\beta$	SE	$\chi^2$	$p$	$\beta$	SE	t-value	$P$	$\beta$	SE	$\chi^2$	$p$
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	<b>0.03</b>	-10.61	1.56	27.23	<0.01	-8.81	3.69	-2.39	<b>0.03</b>	-8.31	1.98	14.24	<0.01	9.00	2.01	4.49	<0.01	8.93	2.09	17.71	<0.01
$T_{a \text{ min}}$	-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	<b>0.05</b>	-6.43	5.51	-1.17	0.26	-4.52	1.98	5.46	<b>0.02</b>	5.70	3.12	1.83	0.09	6.98	2.09	4.91	<b>0.03</b>
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 $T_{a \text{ min}}$	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	<b>0.05</b>	-0.11	0.06	3.29	0.07
Time x $T_{a \text{ min}}$	0.01	0.01	1.10	0.27	0.01	0.003	6.57	<b>0.01</b>	0.01	0.003	2.93	<0.01	0.01	0.002	9.54	<0.01	0.004	0.002	1.98	<b>0.05</b>	0.003	0.001	5.92	<b>0.02</b>
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
$T_{a \text{ min}}$ 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 $T_{a \text{ min}}$ 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 ( $\sigma^7$ ) and female ( $\phi$ ) 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 ( $\sigma^7$ ) and female ( $\phi$ ) 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 ( $\sigma^7$ ) and female ( $\phi$ ) 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 1**

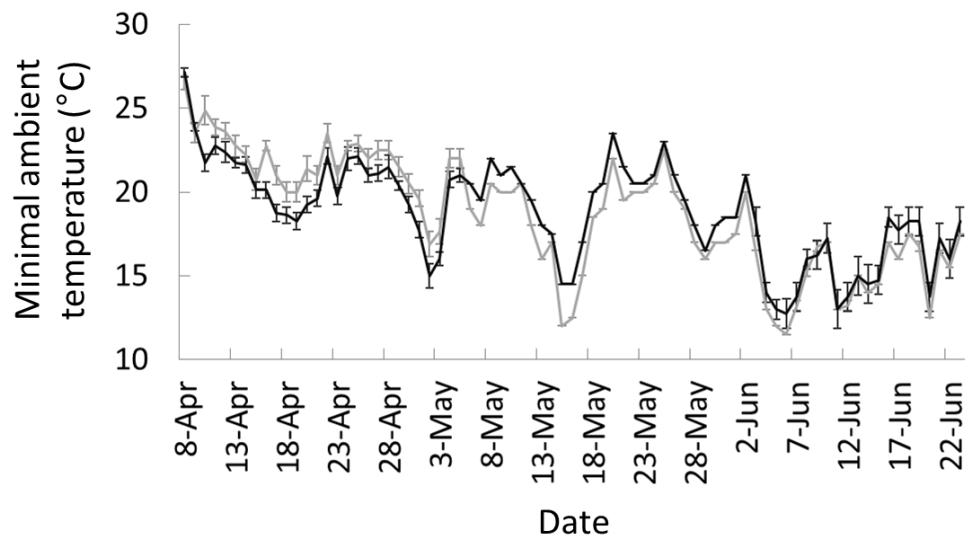
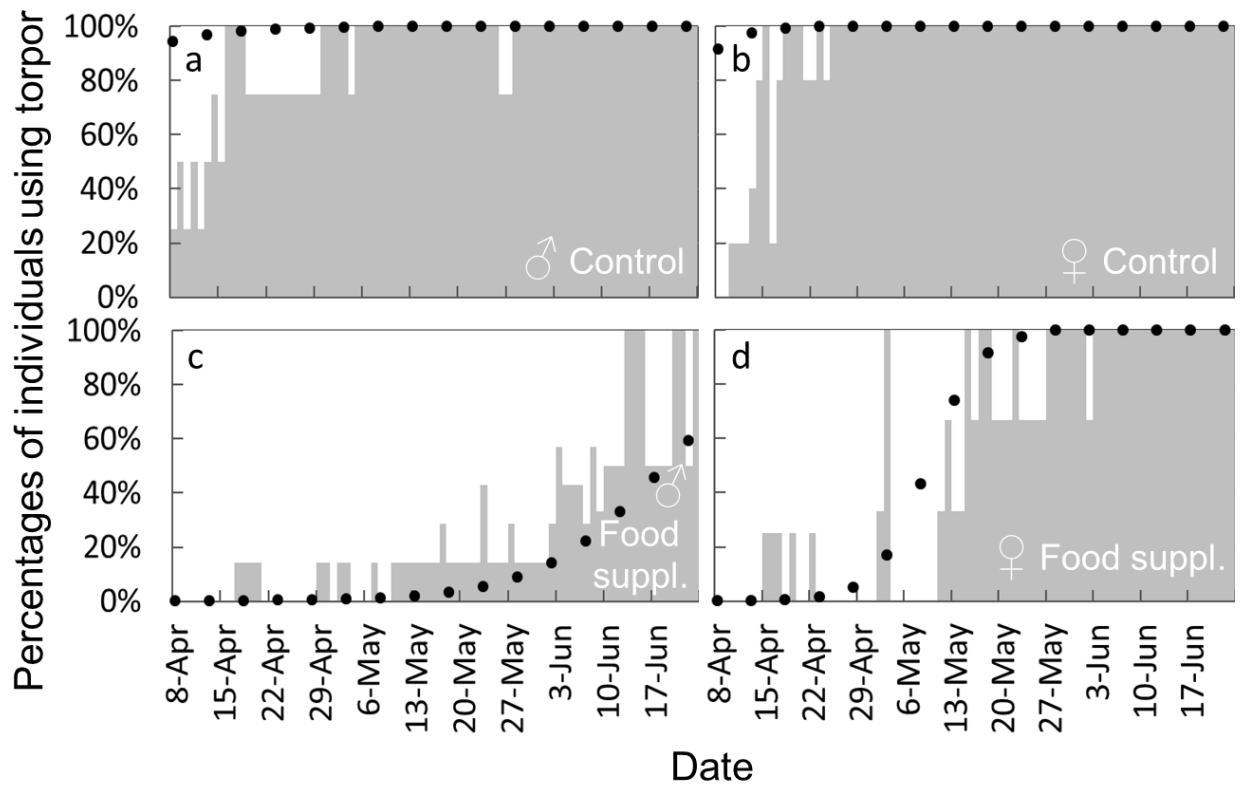


Figure 2



**Figure 3**

