

## RESEARCH ARTICLE

# Extreme physiological plasticity in a hibernating basoendothermic mammal, *Tenrec ecaudatus*

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## ABSTRACT

Physiological plasticity allows organisms to respond to diverse conditions. However, can being too plastic actually be detrimental? Malagasy common tenrecs, *Tenrec ecaudatus*, have many plesiomorphic traits and may represent a basal placental mammal. We established a laboratory population of *T. ecaudatus* and found extreme plasticity in thermoregulation and metabolism, a novel hibernation form, variable annual timing, and remarkable growth and reproductive biology. For instance, tenrec body temperature ( $T_b$ ) may approximate ambient temperature to as low as 12°C even when tenrecs are fully active. Conversely, tenrecs can hibernate with  $T_b$  of 28°C. During the active season, oxygen consumption may vary 25-fold with little or no change in  $T_b$ . During the austral winter, tenrecs are consistently torpid but the depth of torpor may vary. A righting assay revealed that  $T_b$  contributes to but does not dictate activity status. Homeostatic processes are not always linked, e.g. a hibernating tenrec experienced a ~34% decrease in heart rate while maintaining constant body temperature and oxygen consumption rates. Tenrec growth rates vary but young may grow ~40-fold in the 5 weeks until weaning and may possess indeterminate growth as adults. Despite all of this profound plasticity, tenrecs are surprisingly intolerant of extremes in ambient temperature (<8 or >34°C). We contend that while plasticity may confer numerous energetic advantages in consistently moderate environments, environmental extremes may have limited the success and distribution of plastic basal mammals.

**KEY WORDS:** Thermoregulation, Oxygen consumption, Variable body temperature

## INTRODUCTION

Tenrecs are basoendothermic placental mammals primarily from Madagascar that are phylogenetically placed in Afrotheria (Lovegrove, 2012; Everson et al., 2016). Prevailing thought is that a small tenrec species arrived in Madagascar 30–56 MYA and radiated into the remaining extant species (Poux et al., 2008; Everson et al., 2016). This tremendous adaptive radiation resulted in species that are terrestrial, fossorial, arboreal and even aquatic (Garbutt, 1999). Tenrecs have many ‘ancestral’ features that seem even more plesiomorphic than those of the reconstructed

hypothetical placental ancestor *Schrewdinger*. *Schrewdinger* was presumed to lack a cloaca, but to have a large and gyrencephalic brain, zygomatic arches and tympanic bullae, and internal testes (O’Leary et al., 2013). In comparison, tenrecs have a cloaca and, in the case of *Tenrec ecaudatus*, have the smallest brain relative to body mass of all extant mammals (including monotremes and marsupials), are lissencephalic, lack zygomatic arches and tympanic bullae, and have internal testes that are located near the kidneys (Lillegraven and Eisenberg, 1983). These features would suggest that tenrecs may be reminiscent of basal placental mammals.

In Lovegrove’s (2012) plesiomorphic–apomorphic endothermy model, he posited that ancestral mammals were heterothermic and more rigid homeothermy is a derived condition. Lovegrove (2012) defines a basoendotherm simply as any mammal with a body temperature ( $T_b$ ) in the 20th percentile of the frequency distribution of the mammalian  $T_b$  dataset, meaning they must exhibit resting  $T_b$  <35°C. Lovegrove (2012) notes that the endothermic conditions of extant basoendotherms were likely also exhibited by Cretaceous or early Cenozoic mammals. Tenrecs are considered basoendothermic because of the presumed phylogenetic placement of afrotherians and because existing data suggest their  $T_b$  is generally <35°C (Lovegrove, 2012).  $T_b$  is also moderately variable in many of the basal mammalian taxa; some monotremes, marsupials, xenarthrans and afrotherians experience active  $T_b$  that varies by as much as 8°C (Schmidt-Nielsen et al., 1966; McManus, 1969; Scholl, 1974; Gilmore et al., 2000; Oelkrug et al., 2012). The limited existing data on active tenrecs suggest a  $T_b$  always <35°C with moderate variation ( $T_b$ =31.82±0.098°C, mean±s.e.m.; Racey and Stephenson, 1996; Poppitt et al., 1994; Lovegrove et al., 2014, Levesque and Lovegrove, 2014; Levesque et al., 2014).

More pronounced heterothermy in small mammals occurs during hibernation in conjunction with metabolic depression. Importantly, metabolic depression and low  $T_b$  are linked, e.g. it is thought that the metabolic depression allows  $T_b$  to decrease and that the low  $T_b$  may allow depression of metabolic processes (e.g. van Breukelen and Martin, 2015). For instance, hibernating ground squirrels experience decreases of  $T_b$  to as low as –2.9°C (Barnes, 1989) and oxygen consumption rates to as low as 1/100th of active rates. Hibernation is also found in basal mammals including the echidna, some marsupials, and afrotherians like the elephant shrew, golden moles and tenrecs (Ruf and Geiser, 2015; Scantlebury et al., 2008). All five spiny tenrec species, including the focus of this work, *T. ecaudatus*, are known to hibernate (Stephenson and Racey, 1994). The limited data on hibernating *T. ecaudatus* demonstrate that  $T_b$  follows soil temperature for the duration of the hibernation season with no evidence of brief periods of euthermia between bouts of torpor (Lovegrove et al., 2014). The lack of interbout arousals is unique for small hibernators. Interestingly, dwarf lemurs (*Cheirogaleus medius*) will arouse from hibernation if they experience significant periods

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wherein ambient temperature ( $T_a$ ) is  $<30^\circ\text{C}$  (Dausmann et al., 2004; Dausmann et al., 2005). Soil temperature in the *T. ecaudatus* field study was as low as  $\sim 22^\circ\text{C}$  for several months. An interesting question is whether *T. ecaudatus* would arouse if hibernating at an even lower  $T_a$  or whether these tenrecs simply hibernate continuously. If tenrecs are indeed plesiomorphic, perhaps a lack of interbout arousal was an ancestral hibernation characteristic. If so, what may be gleaned from characterizing the physiology of tenrec hibernation?

We established a breeding colony of *T. ecaudatus* in order to examine key parameters affecting their life history in the controlled setting of the laboratory. We found captive tenrecs to exhibit extreme physiological plasticity. Active and hibernating tenrecs may have identical  $T_b$ . Numerous other tenrec life history traits are also highly plastic. We question why such a plastic mammal is not more successful, i.e. if being plastic has selective advantages, then why do the tenrecs not have a more robust global distribution? We provide a surprising explanation that being too plastic may have limited the success and distribution of such mammals in more variable environments. Moreover, by being more precise in their thermoregulation and physiology, the more modern boreoeutherian mammals may have evolved to persist in more variable environments, leading to a wider global distribution.

## MATERIALS AND METHODS

### Acquisition and maintenance of the *T. ecaudatus* colony

Forty wild-caught common tenrecs, *T. ecaudatus* (Schreber 1778), were imported under federal and state permit from Mauritius in June 2014. Approval for this study was granted by the University of Nevada, Las Vegas Institutional Animal Care and Use Committee. Tenrecs were typically maintained on Mazuri insectivore diet (Saint Paul, MN, USA). Tenrecs were supplemented with Purina Puppy Chow (St Louis, MO, USA) and/or Hills A/D diet (Topeka, KS, USA). Tenrecs were weighed regularly to monitor health. All tenrecs were maintained on a 12 h:12 h light:dark cycle. For temperature-controlled experiments, tenrecs were individually housed in rat cages and/or metabolic chambers within an environmental chamber that maintained precise temperature control.

### Measurement and validation of $T_b$

Tenrecs arrived in a torpid state during their hibernation season (austral winter), which precluded surgery to implant temperature loggers. However, we found that  $T_b$  could be reliably and accurately determined through the use of pre-calibrated iButton temperature loggers (DS1922L; Maxim Inc., San Jose, CA, USA) held against the chest by specially designed harnesses. The iButtons were typically set for high-resolution polling ( $\sim 4000$  samples) and sampling frequency typically ranged from 1 to 10 min depending on the desired polling duration. iButton data were downloaded at the end of each experiment period. In an unrelated study, we collected tissue samples from active and torpid tenrecs that were harnessed with chest iButtons at the time of death. We found notable regional heterothermy within the abdomen with as much as  $\sim 3^\circ\text{C}$  variation in temperature depending on probe location (e.g. lower abdomen versus near the liver). In order to allow consistent  $T_b$  measurement, we measured  $T_b$  at the liver with a thermocouple. When tenrecs were housed at a  $T_a$  of 12, 20 or  $28^\circ\text{C}$ , liver temperature ranged from 12.5 to  $30.5^\circ\text{C}$ . When compared with the matching chest temperatures, liver temperature was  $0.467 \pm 0.18^\circ\text{C}$  (mean  $\pm$  s.e.m.) higher ( $P < 0.05$ , paired  $t$ -test,  $N = 21$ ;  $r^2 > 0.99$  in Fig. S1A). These results may be expected as liver temperature in other mammals is slightly higher than other indicators of core  $T_b$  (Dewasmes et al., 2003). Nevertheless, *T. ecaudatus* chest temperature reliably and accurately reflects  $T_b$ .

### Respirometry

Aerobic respirometry (oxygen consumption,  $\dot{V}_{\text{O}_2}$ , and carbon dioxide production,  $\dot{V}_{\text{CO}_2}$ ) was measured with the Promethion Continuous Metabolic Phenotyping System and ExpeData software (Sable Systems International, Las Vegas, NV, USA). The Promethion system auto-baselines for 30 s every 15 min with environmental air, otherwise sampling from each metabolic chamber every second. Metabolic chambers were constructed from Lettuce and Produce Keeper containers ( $\sim 1.8$  l; Container Store, Coppell, TX, USA). Flow rates were  $500 \text{ ml min}^{-1}$ . Preliminary analyses revealed tenrecs to display three physiological states: hibernation, facultative torpor and active. During the austral winter, tenrecs were hibernating and showed the typical signs of torpor, e.g.  $T_b$  approached  $T_a$  and tenrecs were lethargic even when disturbed. We noted that upon entry into the metabolic chamber,  $T_b$  would sometimes slowly increase by  $1\text{--}6^\circ\text{C}$  for various periods (taking up to 3–4 h to peak with a similar period to return to the original value) in what appeared to be a partial thermal arousal. Importantly, these tenrecs maintained a lethargic state typical of torpor and never aroused to an active state. Furthermore, at  $T_a < 20^\circ\text{C}$ , tenrecs were noted to experience 30–45 min apneic periods. To avoid sampling bias caused by the partial thermal arousals and the apneic periods, data for hibernating tenrecs represent an 8 h period of stable  $T_b$  (after  $\sim 14$  h of a typical 24 h assay). During the active season (after the resumption of feeding, i.e. October to April), tenrecs were also sometimes facultatively torpid wherein  $T_b$  approached  $T_a$  and animals displayed lethargy typical of torpor. Care was taken to gently place the tenrecs into the metabolic chamber as these animals were prone to disturbance and would occasionally arouse from the facultative torpor into an active state (in contrast to hibernators, which remained consistently lethargic). Analyses were restricted to 90 min for these assays because of the lability of facultative torpor. Active-season tenrecs that demonstrated no signs of lethargy had remarkably variable resting  $\dot{V}_{\text{O}_2}$  and  $T_b$ . To best illustrate this variability, the analyses comprised a 60 min period with either the highest or lowest stable  $\dot{V}_{\text{O}_2}$ . We defined stable as a period where there was little variability that may have indicated obvious activity or movement. The chamber was small enough to prevent the animals from exercising. Each analysis group consisted of eight tenrecs except for facultative torpor at  $28^\circ\text{C}$ , where only seven tenrecs were available for the analysis. All  $\dot{V}_{\text{O}_2}$  data are presented as  $\text{ml O}_2 \text{ g}^{-1} \text{ body mass h}^{-1}$ . Box plots were generated using standard box plot parameters wherein the box identifies the first quartile (bottom line), median (middle line) and third quartile (top line) of the dataset; the whiskers represent the maximum and minimum values for each dataset. In order to best demonstrate the extreme variability in *T. ecaudatus* aerobic metabolism, we chose to omit outlier analyses and all data are included.

### Electrocardiogram (ECG) measurement and heart rate analysis

ECGs were collected through use of a three-lead ECG module, iWorx Data Recorder and LabScribe (IWX-214 and LabScribe v3; iWorx Systems Inc., Dover, NH, USA). Heart rate ( $\text{beats min}^{-1}$ ) was determined for a minimum of three consecutive minutes every 10 min of the analysis period.

### Righting response time (RRT) assay

Tenrecs ( $N = 8$ , except for the April assay at  $28^\circ\text{C}$ , wherein 7 tenrecs were used because of an unexpected mid-trial death) were housed at 12, 20 or  $28^\circ\text{C}$  for a minimum of 24 h prior to the righting trials. To avoid potential bias from disturbance, the order in which animals

were used for each trial was randomized through use of a random number generator. We found no correlation between this order and RRT. Each trial followed strict timing protocols throughout the year, e.g. animals were placed into the apparatus within 20 s of removal from the environmental chamber and there were 3 min intervals between animals. Animals were placed on their backs in a cradle and allowed 60 s to attempt to right themselves. Righting was video recorded and the time to right was determined from the video (Movies 1 and 2).

### Emergence analysis

Most hibernators experience marked changes in  $T_b$  at the cessation of the hibernation season as active behaviors resume. However, even active tenrecs have variable  $T_b$ . As a result, we used body mass changes as tenrecs resumed eating following emergence from hibernation to estimate date of emergence.

### Statistics

As appropriate, data were analyzed using ANOVA with subsequent Fisher's least significant difference (LSD) *post hoc* analyses or paired and unpaired Student's *t*-test. Statistical differences were assumed when  $P < 0.05$ . A Bonferroni adjustment in Fig. 2 was used because of the large number of comparisons. All data presented with error calculations (e.g.  $\dot{V}_{O_2}$ , mass analysis, etc.) are given as means  $\pm$  s.e.m. unless otherwise noted.

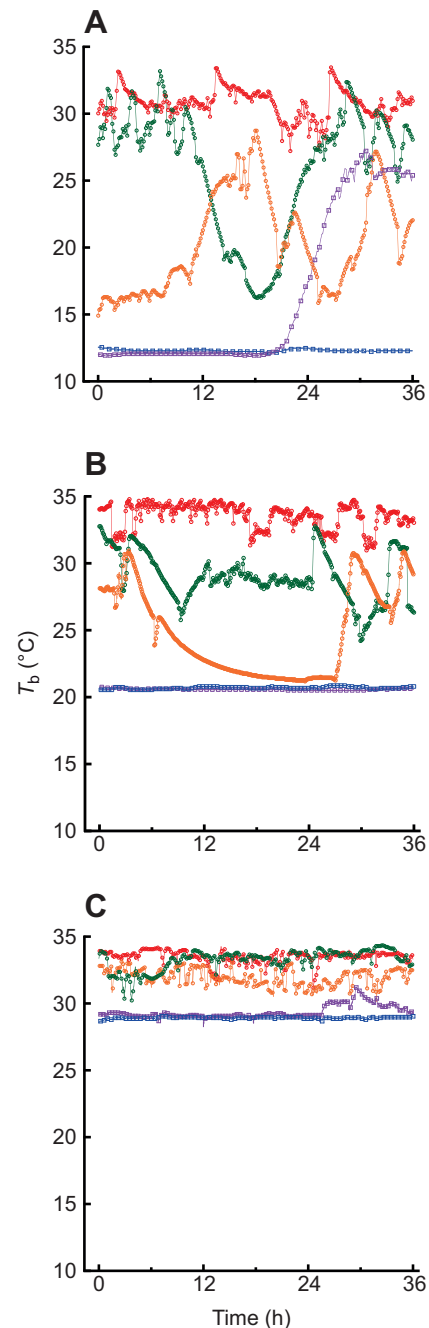
## RESULTS

### $T_b$

$T_b$  in captive tenrecs was extremely plastic during both hibernation and the active season (Fig. 1). During the austral winter, tenrecs can seasonally hibernate at low or high  $T_a$  from at least 12 to 28°C (Fig. 1). During hibernation, tenrec  $T_b$  approximates  $T_a$  and tenrecs are consistently lethargic. Importantly, when housed at 12°C and disturbed,  $T_b$  of hibernating tenrecs was noted to increase only  $\sim 1$ –6°C. Remarkably, active tenrecs may also experience  $T_b$  ranging from  $\sim 12$  to 34°C that frequently waxes and wanes throughout the day. Even with a  $T_b$  of  $\sim 12.5$ °C, these animals display no lethargy or ataxia and are capable of running and swimming. Although active tenrec  $T_b$  is oftentimes quite variable throughout the day,  $T_b$  may also be relatively stable for shorter periods of time (Fig. S1B–D). Not surprisingly, the differences between  $T_b$  and  $T_a$  decreased as  $T_a$  increased, e.g. animals maintained at a  $T_a$  of 28°C had less variation in  $T_b$  than animals maintained at a  $T_a$  of 12°C. Active-season tenrecs were also capable of using torpor facultatively at a  $T_a$  of 12, 20 and 28°C (Fig. 1). Active-season tenrecs that had resumed active behaviors, e.g. eating and reproduction, were considered to be in facultative torpor when the tenrec became lethargic and  $T_b$  approximated  $T_a$  for several days. In contrast to hibernating animals, facultatively torpid tenrecs were sensitive to disturbance wherein animals would arouse into a fully active and alert state with no indication of lethargy or ataxia.

### Metabolism

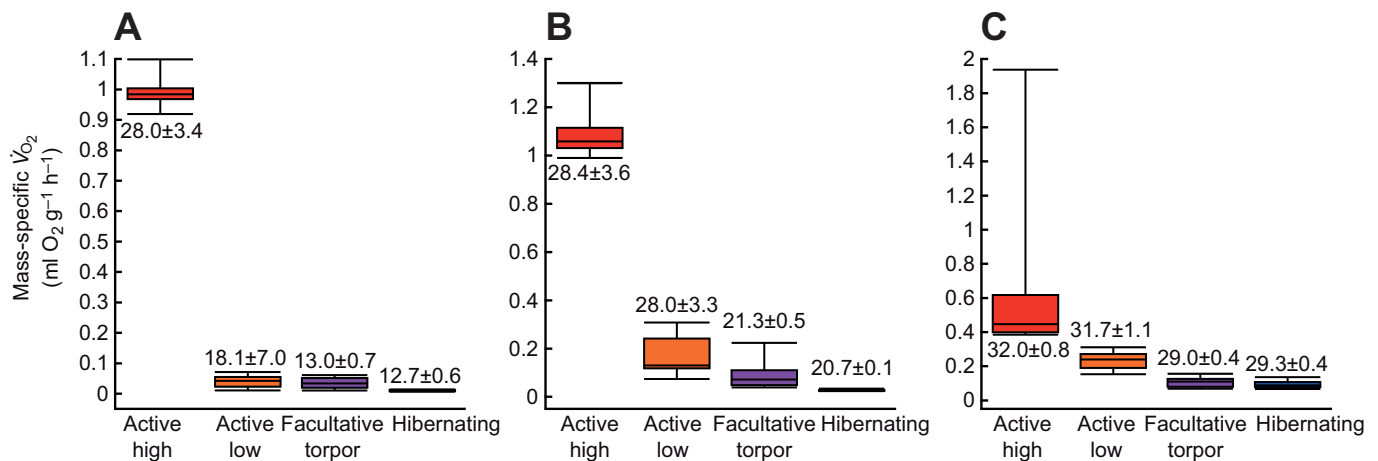
Metabolism in active tenrecs may vary by 25-fold (Fig. 2). In order to best demonstrate this extreme variability, 60 min periods of the highest (active high) and lowest (active low) stable respirometry data are presented in Fig. 2. Oxygen consumption rate in active tenrecs did not simply switch between high and low levels, but rather occurred along a continuum of rates between the active high and active low levels. Tenrec  $\dot{V}_{O_2}$  was only partially dependent on  $T_b$ . For instance, the active low  $\dot{V}_{O_2}$  in Fig. 2A was not statistically different from the  $\dot{V}_{O_2}$  of facultative torpid or hibernating tenrecs



**Fig. 1. Extreme variability of body temperature ( $T_b$ ) of *Tenrec ecaudatus*.** Tenrecs were housed at an ambient temperature ( $T_a$ ) of (A) 12°C, (B) 20°C or (C) 28°C. Note the lack of homeothermy in active tenrecs (red, green and orange circles). Individual active tenrecs may have low or high  $T_b$  that can change  $>2.5$ °C  $h^{-1}$ . Alternatively, active-season tenrecs may spontaneously enter periods of facultative torpor (purple squares) where  $T_b$  approximates  $T_a$ . During the austral winter, tenrecs maintain a constant state of hibernation (blue squares).

despite these active tenrecs having higher  $T_b$  (ANOVA,  $P > 0.05$ ). Tenrecs may also have similar high  $\dot{V}_{O_2}$  ( $\sim 1$  ml  $O_2$   $g^{-1}$   $h^{-1}$ ) and  $T_b$  ( $\sim 28$ °C) even when there is an 8°C difference in  $T_a$  ( $T_a$  of 12 or 20°C; Fig. 2A,B). Additional examples of how metabolism may be disconnected from  $T_b$  in individual tenrecs are provided in Figs S2 and S3.

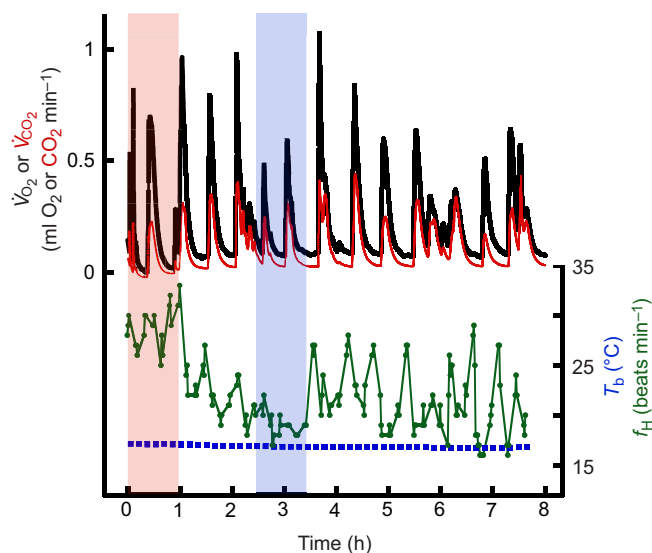
When all data were analyzed together, no differences in  $\dot{V}_{O_2}$  were found between the hibernating, facultative torpid or even the active



**Fig. 2. Plastic oxygen consumption ( $\dot{V}_{O_2}$ ) in tenrecs.**  $T_a$  was (A) 12°C, (B) 20°C or (C) 28°C. Data represent box plots of the highest (active high) or lowest (active low)  $\dot{V}_{O_2}$  in non-torpid active-season tenrecs (see Materials and Methods,  $N=8$ ). For comparison, data from active-season facultatively torpid tenrecs and hibernating tenrecs are included. Note, aerobic metabolism is not necessarily related to  $T_b$  or  $T_b - T_a$  differentials, e.g. active high values for 12 and 20°C are similar. All active high groups showed significantly greater  $\dot{V}_{O_2}$  than all other comparisons. No other differences were detected when all groups were analyzed together ( $P < 0.05$ ; ANOVA). Numbers associated with the boxes represents  $T_b$  (°C; means  $\pm$  s.d.).

low state at  $T_a$  of 12, 20 or 28°C (Fig. 2; ANOVA,  $P > 0.05$ ). Only when active animals were removed from the analyses (i.e. reducing the number of comparisons by comparing only facultative torpid and hibernating tenrecs) did statistical differences between temperatures become evident; in these comparisons,  $\dot{V}_{O_2}$  decreased with decreasing temperature as expected ( $Q_{10}=3.7$  for hibernating tenrecs and 2.0 for facultatively torpid tenrecs between 12 and 28°C; ANOVA,  $P < 0.05$ ).

Both active and torpid tenrecs experienced extended periods wherein there were unexpected relationships between  $T_b$ ,  $\dot{V}_{O_2}$  and heart rate. For instance, identical  $\dot{V}_{O_2}$  values were accommodated by heart rates that varied by 34% (Fig. 3). Additional examples of metabolic flexibility are provided in Figs S2 and S3.



**Fig. 3. Demonstration of the lack of coordination of basic homeostatic mechanisms.**  $T_a$  was 16°C. Torpid  $\dot{V}_{O_2}$  was the same in the two highlighted (red and blue) 60 min periods (0.171 ml O<sub>2</sub> min<sup>-1</sup>). However, while heart rate ( $f_H$ ) in the red phase averaged 28.8  $\pm$  2.0 beats min<sup>-1</sup>, heart rate during the blue phase averaged 19.0  $\pm$  1.3 beats min<sup>-1</sup>, representing a ~34% decrease in heart rate despite identical  $\dot{V}_{O_2}$ . Note, the 30–45 min periods between breaths in this hibernating tenrec.

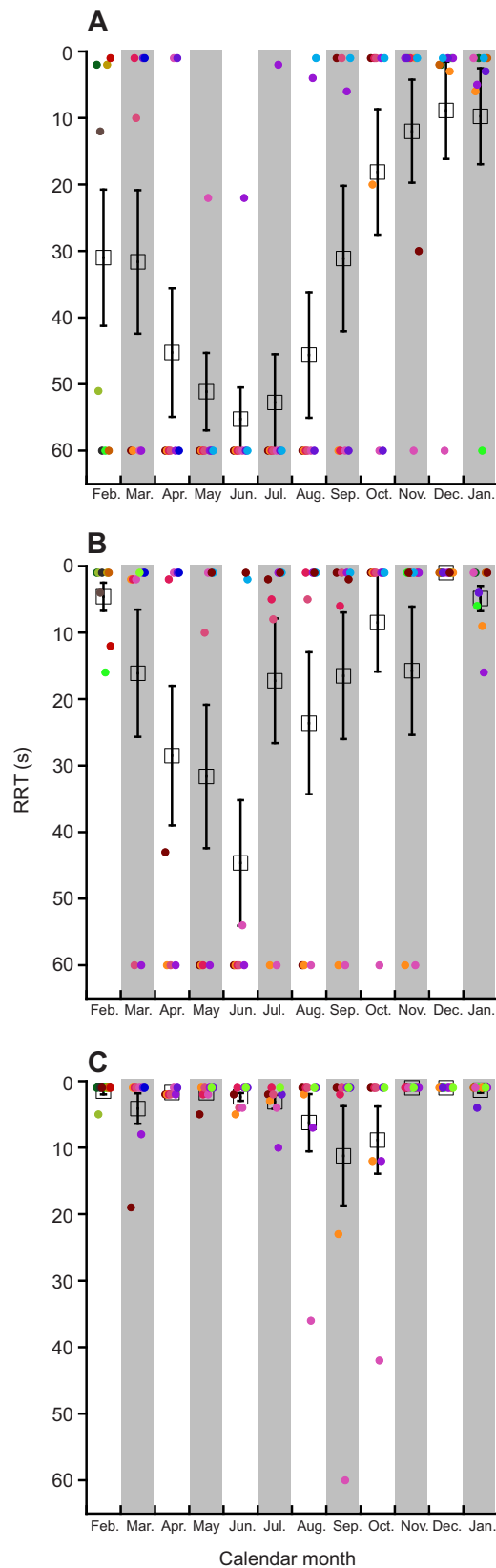
### Righting response

As indicated above and unlike in other hibernators, we observed that  $T_b$  was a poor indicator of metabolic status. We noted that hibernating tenrecs, unlike common hibernator models such as ground squirrels, were not simply torpid or non-torpid during the hibernation season. Rather, early season hibernating tenrecs typically appeared ‘less’ torpid and more responsive to disturbance, mid-season hibernators were deeply torpid and resisted disturbance, and late season hibernators were less torpid. Furthermore, we found that  $T_b$  also partially predicted hibernation depth, e.g. a cold hibernating tenrec was more likely to be profoundly lethargic and a warm tenrec was more likely to be partially ambulatory and less ataxic when handled. Importantly, hibernating tenrecs were always somewhat lethargic and ataxic regardless of  $T_a$ . In order to best illustrate annual torpor use and this concept of ‘more or less’ torpid, we monitored lethargy in *T. ecaudatus* throughout the year through the use of a righting response assay (Fig. 4; Movies 1 and 2). In general, increased lethargy occurred when animals were housed at a lower  $T_a$ , in both active and hibernation seasons. Tenrecs housed at a  $T_a$  of 12°C were usually less likely to right themselves within the 60 s assay while tenrecs housed at a  $T_a$  of 28°C rarely spent more than a few seconds righting themselves. Hibernating tenrecs at a  $T_a$  of 28°C were very readily ‘aroused’ and although they were able to right quickly, we still noted some ataxia in these animals. Interestingly, some animals with  $T_b$  near  $T_a$  to as low as 12°C were able to right themselves quickly. Conversely, some warm animals took the full 60 s to right (Fig. S4). At a  $T_a$  of 12°C, a defined seasonality was present wherein *T. ecaudatus* appeared most lethargic in June–July and most active in December, consistent with our observations of the hibernation and active seasons (Fig. 4A).

### Emergence from hibernation

Unlike in other hibernators, the end of the hibernation season in tenrecs was a gradual transition and occurred over a couple of weeks. As individual tenrecs transitioned out of hibernation, they slowly became less lethargic, less ataxic and more aware of their surroundings (L.S., M.D.T. and F.v.B., personal observations). Tenrecs then resumed active-season behaviors like eating, growth and reproduction. By tracking food intake and body mass, approximate dates of emergence from hibernation for each tenrec





**Fig. 4. Demonstration of seasonal and  $T_a$  effects on righting response time (RRT).** Tenrecs were housed at a  $T_a$  of (A) 12°C, (B) 20°C or (C) 28°C. Filled circles of different colors represent individual tenrecs. Squares represent means  $\pm$  s.e.m. of that month's RRT. Seasonality was more evident in tenrecs housed at 12°C than at 28°C; however, even when housed at 28°C, some animals displayed a RRT of >20 s.

were determined (Fig. 5). Some tenrecs emerged as early as August or as late as January, with most tenrecs having exited hibernation by October or November (Fig. S5A).

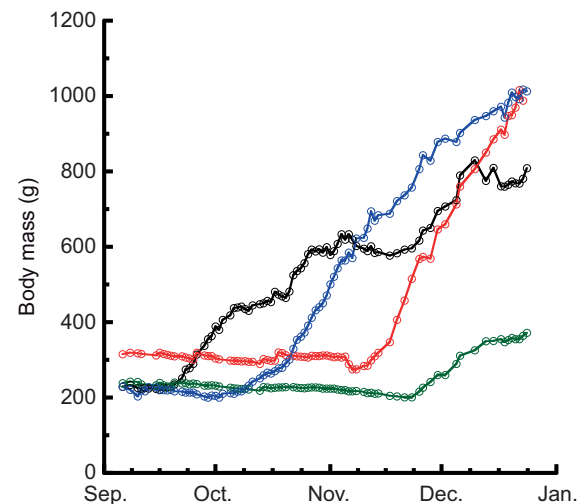
### Growth rates

To establish our colony, we obtained wild-caught juvenile and adult tenrecs that were generally small ( $262 \pm 15.9$  g;  $N=35$ ). Upon emergence from hibernation, these tenrecs ate copious amounts of food and grew tremendously in a short period of time (2.5- to 3-fold in  $\sim 4$  months; average maximal daily mass gain of  $44.6 \pm 4.0$  g day $^{-1}$ ;  $N=35$ ; Fig. 5). This growth was not simply the result of fat accumulation as these animals also grew in scale. Furthermore, dramatic growth was not limited to juveniles as adult tenrecs also experienced similar growth (Fig. S5B).

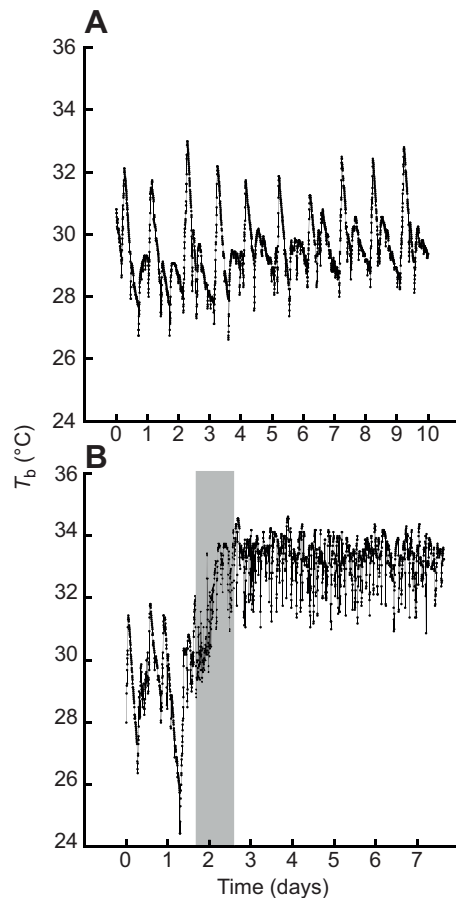
Despite a birth mass of  $12.15 \pm 0.36$  g ( $N=31$ ; four litters), captive tenrecs oftentimes weigh >400 g by the time they wean at 5 weeks. We observed juvenile tenrecs eating from their mothers' mouths and/or eating soft foods during the first week post-partum. The profound growth is not simply a function of tenrecs growing as quickly as possible as there may be surprising consistency of growth rates within a given litter (Fig. S5C,D). Further, there may be multiple growth rate trajectories represented within a given litter, e.g. some tenrecs may grow quickly while others may grow more slowly. Although most of our tenrecs grew quickly prior to the hibernation season, some juveniles entered hibernation at a body mass less than 150 g. These smaller tenrecs experienced the same profound post-hibernation growth as our wild-caught tenrecs.

### $T_b$ in pregnant and post-partum tenrecs

Prior to parturition, tenrec  $T_b$  may be variable during gestation (Fig. 6; Fig. S6). Following parturition, females experienced increased and more stable  $T_b$  (above 30°C; Fig. 6; Fig. S6). In one extraordinary case, a pregnant female housed at a  $T_a$  of 12°C experienced a >24 h period wherein  $T_b$  was  $13.5 \pm 0.5^\circ\text{C}$  (Fig. S6G). We estimated that she was  $\sim 3$  weeks into her pregnancy.



**Fig. 5. Tenrecs may experience extreme growth following exit from hibernation.** Tenrecs were observed to gradually increase in activity for as much as 2 weeks prior to the onset of very rapid growth and mass gain. Different colors represent individual tenrecs. Average daily maximum mass gain was  $44.6 \pm 4$  g day $^{-1}$ ,  $N=35$ .



**Fig. 6. Tenrecs can invoke periods of higher endothermy/homeothermy.** (A) A non-pregnant tenrec shows typical variation in  $T_b$ . (B) Tenrecs that give birth (shaded area indicates day of parturition) maintain a higher and less variable  $T_b$  post-partum. These tenrecs were housed at room temperature.

## DISCUSSION

Common tenrecs have highly variable  $T_b$  and rates of oxygen consumption (Figs 1–3 and 6; Figs S1–S4 and S6). This variability is not restricted to the hibernation season in tenrecs. Moderately variable active-season  $T_b$  is prevalent in many of the basal mammalian taxa. In monotremes like the echidna *Tachyglossus aculeatus*, active  $T_b$  was  $30.7 \pm 1.03^\circ\text{C}$  with a range of as much as  $4.1^\circ\text{C}$  when held at a  $T_a$  between 0 and  $25^\circ\text{C}$  (Schmidt-Nielsen et al., 1966). In a marsupial, the opossum *Didelphis virginiana*,  $T_b$  was typically  $33\text{--}35^\circ\text{C}$ , with the lowest recorded  $T_b$  reaching  $30.8^\circ\text{C}$  when held at a  $T_a$  from  $-10$  to  $32^\circ\text{C}$  (McManus, 1969). In a xenarthran, the brown-throated three-toed sloth, *Bradypus variegatus*, active  $T_b$  ranged from  $28$  to  $35^\circ\text{C}$  with the circadian cycle (Gilmore et al., 2000). Of note is that these ranges for  $T_b$  are relatively moderate, with few animals experiencing variations of more than  $8^\circ\text{C}$ . Even in other afrotherians like elephant shrews,  $T_b$  varied by only a few degrees during the active season (Mzilikazi and Lovegrove, 2004; Mzilikazi and Lovegrove, 2005; Oelkrug et al., 2012). We found  $\sim 20^\circ\text{C}$  variation in  $T_b$  in both active and hibernating tenrecs.

In *T. ecaudatus*, active-season aerobic metabolism (active low) may be statistically comparable to that of even torpid tenrecs and independent of a reduction in  $T_b$  (Fig. 2). Conversely, oxygen consumption in active tenrecs may also be  $\sim 25$ -fold higher (active high). For instance, at a  $T_a$  of  $12^\circ\text{C}$ , stable  $\dot{V}_{\text{O}_2}$  across a 60 min period in active tenrecs was as low as  $0.0405 \pm 0.021 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$

(active low) or as high as  $0.9916 \pm 0.052 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (Fig. 2). Surprisingly,  $T_b$  did not dictate metabolism. Tenrecs housed at 12 and  $20^\circ\text{C}$  maintained similar  $\dot{V}_{\text{O}_2}$  and  $T_b$ , e.g. active high  $\dot{V}_{\text{O}_2}$  across a 60 min period for animals maintained at a  $T_a$  of  $20^\circ\text{C}$  was  $1.0856 \pm 0.097 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  despite these animals having the same  $T_b$  ( $\sim 28^\circ\text{C}$ ) as the active high animals that were maintained at  $12^\circ\text{C}$ . Remarkably,  $T_b$  of the active low animals housed at  $20^\circ\text{C}$  was also  $\sim 28^\circ\text{C}$ , despite these tenrecs having a  $\sim 6.4$ -fold lower  $\dot{V}_{\text{O}_2}$  than that of the active high animals. We note that active low metabolism is not simply torpor use.  $T_b$  was higher than  $T_a$  in active low tenrecs. Furthermore, active low tenrecs did not exhibit the lethargy and ataxia of facultatively torpid tenrecs found during the active season. Although the data presented here do not offer a mechanistic basis, it appears tenrecs may be able to modulate heat retention and conductance independent of other physiological parameters like  $\dot{V}_{\text{O}_2}$ . An alternative explanation for the lack of a relationship between  $\dot{V}_{\text{O}_2}$  and  $T_b$  is that there could be a significant anaerobic component to metabolism. The data for  $\dot{V}_{\text{CO}_2}$  generally coincide with those for  $\dot{V}_{\text{O}_2}$  and do not support the use of anaerobic metabolism. Further, identical  $T_b$  and  $\dot{V}_{\text{O}_2}$  values could be accommodated by heart rates that vary by 34% (Fig. 3). These data support a notion that metabolism in tenrecs may simply be plastic in comparison to  $T_b$ . In common tenrecs, the large variation in metabolism is not associated with the use of torpor or hibernation.

In most mammalian species, such profound changes in  $T_b$  and metabolism would be associated with the use of torpor or hibernation (Ruf and Geiser, 2015; van Breukelen and Martin, 2015). Torpor is traditionally defined as a reduction in metabolism to levels below what is required to maintain normal metabolic activities. In many cases, torpid  $T_b$  may approach  $T_a$  and is associated with extreme metabolic depression. In other cases, a more moderate depression of metabolism results in torpid  $T_b$  being higher than  $T_a$ . The depressed metabolism serves as a mechanism to conserve energy during times of limited resource availability or environmental stress. Active suppression of metabolism has been previously demonstrated in hibernating black bears (Tøien et al., 2011). Upon exit from hibernation, bear  $T_b$  increases by  $\sim 2^\circ\text{C}$ . However,  $\dot{V}_{\text{O}_2}$  slowly increases over the ensuing 2–3 weeks. In tenrecs, remarkable metabolic suppression is seen during facultative torpor and hibernation. For instance, hibernating tenrec  $\dot{V}_{\text{O}_2}$  at  $12^\circ\text{C}$  is  $0.0115 \pm 0.023 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ . For comparison, hibernating Arctic ground squirrel  $\dot{V}_{\text{O}_2}$  at  $4^\circ\text{C}$  is  $0.012 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (Buck and Barnes, 2000). Interestingly, euthermic ( $T_b \sim 36^\circ\text{C}$ ) Arctic ground squirrel  $\dot{V}_{\text{O}_2}$  at a  $T_a$  of  $2^\circ\text{C}$  approximates our active high  $\dot{V}_{\text{O}_2}$  at a  $T_a$  of 12 and  $20^\circ\text{C}$  (Karpovich et al., 2009), indicating that tenrecs are not simply hypometabolic. It appears that tenrecs are able to suppress aerobic metabolism to a degree equal to that of the most competent hibernators despite being at least  $8^\circ\text{C}$  warmer.

In all described mammalian hibernators to date, the hibernation phenotype consists of bouts of torpor interrupted by brief periodic returns to euthermia wherein critical homeostatic processes are restored (van Breukelen and Martin, 2015). Lovegrove et al. (2014) found that wild *T. ecaudatus* did not experience these interbout arousals. Common tenrecs hibernate in small, underground burrows  $\sim 1 \text{ m}$  deep that are sealed to the outside with soil for the 8–9 month hibernation season (Lovegrove et al., 2014; Rand, 1935). Recent video evidence showed 13 torpid tenrecs were removed from a single hibernaculum by hunters, suggesting tenrecs may hibernate socially ([https://youtu.be/w8OAm\\_eeNd8](https://youtu.be/w8OAm_eeNd8)). Soil temperatures in the Lovegrove et al. (2014) field study were never  $< 22^\circ\text{C}$ , which precluded colder tenrec  $T_b$ . Data for fat-tailed dwarf lemurs, *Cheirogaleus medius*, demonstrate that periodic interbout arousals

occur only if the tree hole is  $<30^{\circ}\text{C}$  during its hibernation season (Dausmann et al., 2004). An important question is whether lower  $T_b$  (i.e.  $T_b < 22^{\circ}\text{C}$ ) during hibernation would elicit the use of periodic arousals in common tenrecs (van Breukelen and Martin, 2015). When held at a  $T_a$  between 12 and  $28^{\circ}\text{C}$  during the hibernation season, we found captive tenrecs had a core  $T_b$  that closely approximated  $T_a$  (Fig. 1; Figs S1B–D, S2) and found no evidence of natural periodic interbout arousals even at a  $T_b$  as low as  $\sim 12^{\circ}\text{C}$ . Upon disturbance, some tenrecs experienced  $\sim 1\text{--}6^{\circ}\text{C}$  increases in  $T_b$  that may last from  $<1$  h to 14 h. We noticed these partial thermal arousals occurred in close proximity to the daily checks or weighings. Tenrec thermal arousals are highly variable and lack predictability, features inherent to canonical interbout arousals. If the function of an interbout arousal is to allow re-establishment of homeostatic processes with high  $T_b$  (van Breukelen and Martin, 2001), it is difficult to see how a modest increase in  $T_b$ , e.g.  $12.5\text{--}15^{\circ}\text{C}$ , would accomplish those functions. Further, these same partial thermal arousals occurred at a  $T_a$  of  $28^{\circ}\text{C}$  where no similar need could be rationalized. As a result, we contend that the periods of increased  $T_b$  in captive tenrecs do not represent a natural feature of tenrec hibernation and should not be considered canonical interbout arousals. Instead, we liken these partial thermal arousals to unsuccessful alarm arousals. In ground squirrels, handling of torpid animals prematurely induces an interbout arousal where  $T_b$  fully returns to euthermic levels for the prescribed 12–20 h period before continuing the next torpor bout (alarm arousal; Utz and van Breukelen, 2013). Importantly, not all manipulations cause alarm arousals. In some squirrels,  $T_b$  may increase  $\sim 1\text{--}2^{\circ}\text{C}$  before returning to the pre-handling  $T_b$ . In our experience with ground squirrels, we have never seen a partial arousal where the increase in  $T_b$  was greater than  $2^{\circ}\text{C}$ . Tenrecs never fully arouse from torpor during the hibernation season and there is always some indication of lethargy and ataxia. This continuous lethargy/torpor may be demonstrated by examination of heart rates. During the active season, heart rate was  $151.3 \pm 10.8$  beats  $\text{min}^{-1}$  at room temperature (data not shown;  $N=7$ ). We handled tenrecs while they were hibernating at room temperature. Heart rate increased during handling to as high as 87 beats  $\text{min}^{-1}$  ( $71.2 \pm 5.15$  beats  $\text{min}^{-1}$ ;  $N=5$  tenrecs) but quickly returned to values of  $32.6 \pm 2.04$  beats  $\text{min}^{-1}$  within  $94.4 \pm 35.7$  s. Even with the reduced heart rate, some tenrecs were able to ambulate (albeit these tenrecs were ataxic).

To better understand this more or less torpid concept, we employed a RRT assay (Fig. 4). Our assay data confirmed our observations that hibernation in tenrecs represents a continuum, with animals being most torpid in June during the austral winter (Fig. 4A). We found  $T_b$  to be a less than reliable indicator of metabolic status.  $T_b$  partially predicts activity status, with warmer tenrecs being able to right themselves more quickly. However, not all tenrecs were predictable, e.g. one tenrec with a  $T_b$  of  $29.5^{\circ}\text{C}$  experienced a 30 s RRT despite being housed at  $12^{\circ}\text{C}$  (Fig. S4).

Torpid tenrecs ( $T_b < 20^{\circ}\text{C}$ ) experienced repeated cycles of relatively short, single-peak bursts followed by as much as 45 min of waning oxygen consumption (Fig. 3; Fig. S2). The ventilation pattern was reminiscent of insect discontinuous gas exchange (Lighton, 1998). This mechanism is proposed to allow efficient gas exchange in subterranean environments that become hypoxic and hypercapnic. However, our experiments did not involve exposure to hypoxia or hypercapnia. One possible function of these brief bursts of metabolic activity may be that they serve the same role as a canonical interbout arousal. For instance, one might envisage periodic increases in heart rate as in Fig. 3 that may lead to

higher blood pressure and pulses in kidney function without increases in  $T_b$ . We found that tenrecs hibernating at a  $T_a$  of  $12^{\circ}\text{C}$  are able to urinate (M.D.T., L.S. and F.v.B., personal observations). In ground squirrels, kidney function is restricted to the interbout arousal (Jani et al., 2013). Our laboratory is currently investigating these ideas.

In common models of hibernation like arctic ground squirrels, the timing of immergence and emergence from hibernation is generally synchronized and highly predictable to within a few days (Sheriff et al., 2011). In *T. ecaudatus*, the timing seems much more plastic. We noted that tenrecs slowly exited hibernation and became more aware of their surroundings and less lethargic over the course of  $\sim 2$  weeks. Tenrecs then resumed active-season behaviors, e.g. rapid and coordinated movement, eating, voiding of waste products, and reproduction. The timing of emergence of our captive tenrec population varied by  $\sim 5$  months (Fig. 5; Fig. S5A). We have maintained the colony since 2014 and have observed no indications that the annual cycle is shifting from a southern towards a northern hemisphere cycle. Tenrecs immerged into hibernation as early as January and in some exceptional cases as late as July (data not shown). Most tenrecs were hibernating by May.

Plastic growth rate trajectories were evident in neonatal, juvenile and adult tenrecs. While the profound mass gain in neonatal tenrecs is not as extreme as the  $\sim 25\%$  daily mass gain in neonatal hooded seals, *Cystophora cristata*, which wean at  $\sim 3\text{--}5$  days of age, the mass gain in these seals is mostly fat (Bowen et al., 1985), whereas in tenrecs the mass gain reflected changes in skeletal dimension. Interestingly, profound growth even occurred in adult tenrecs. Indeterminate growth is considered the ancestral condition in amniotes (Hariharan et al., 2016); during indeterminate growth, increases in the length of the major body axis occur after the animal is thought to have reached a mature size. That our wild-caught adult tenrecs grew  $2.5 \pm 0.37$ -fold suggests a degree of indeterminate growth that appears unique in the mammalian world (Hariharan et al., 2016).

Tenrecs mated upon emergence from hibernation ( $\sim$ September to November). Little is known of the reproductive biology of tenrecs other than that it seems to be plastic. In the lesser hedgehog tenrec, *Echinops telfairi*, gestation is reported to vary between 50 and 79 days (Künzle et al., 2007). Gestation length in *T. ecaudatus* is suggested to be 58–64 days (Eisenberg, 1975). We have observed tenrecs giving birth as early as November and as late as February; however, we are not familiar with any studies indicating how factors like lowered and variable  $T_b$  and/or use of facultative torpor might affect gestation length. Interestingly, pregnant tenrecs were found to display extremely variable  $T_b$  (as low as  $13.5 \pm 0.5^{\circ}\text{C}$ ) with little apparent effect on their reproductive success (Fig. S6G; Nicoll, 1982).

Common tenrec litter size may be enormous (as many as 32 babies), although litter sizes of 16–20 are more typical (Nicoll and Racey, 1985). Our colony's litter sizes were generally smaller, with a maximum of 19. Superfetation has been suggested in tenrecs (Poduschka, 1996). This ability to simultaneously support multiple litters at different developmental stages in the uterus is well documented in European hares (Roellig et al., 2011). In our limited experience, one tenrec was determined to be pregnant, isolated from further interactions with males, gave birth in isolation and was subsequently maintained in isolation for 40 days before giving birth to another litter. This occurrence suggests that, although rare, tenrecs may avail themselves of superfetation. A combination of large litter size and superfetation would suggest tremendous reproductive output is available to these tenrecs.



Endothermy in modern placental mammals is widespread. Although there are several tenable models for why endothermy may have evolved, all predict selective advantages to animals that maintain warmer and more stable  $T_b$  (Lovegrove, 2016). Are tenrecs simply not able to maintain a warmer and more stable  $T_b$ ? We found female tenrecs became increasingly endothermic and homeothermic on the day of parturition (Fig. 6; Fig. S6). These data suggest that while tenrecs are normally very plastic and display highly variable  $T_b$ , they may experience periods wherein increased endothermy/homeothermy is possible. While *T. ecaudatus* display this increased endothermy/homeothermy post-parturition, work with other species of tenrec (*E. telfairi* and *Setifer setosus*) suggests increased homeothermy while tenrecs are pregnant or across the breeding season in males (Oelkrug et al., 2013; Levesque and Lovegrove, 2014).

If increased endothermy/homeothermy were advantageous and given these tenrecs are capable of increased endothermy/homeothermy, then why not be more consistently homeothermic? The answer may lie in the advantages of being plastic. Many consider canonical mammalian hibernation as a means of passing the winter when resources are low. Tenrecs may simply be exploiting hibernation-like strategies in their active-season physiology. Varying metabolism and  $T_b$  would presumably allow for the conservation of energetic stores. One can then flip this argument around and ask why a placental mammal would be less plastic. That answer may lie in the limits to plasticity. Despite being considered a hypervariable environment, much of Madagascar is rather warm (Ohba et al., 2016). In fact, Madagascar's climate is thought to have changed very little since tenrecs first colonized the island 30–56 MYA (Everson et al., 2016; Ohba et al., 2016). We found tenrecs appear highly stressed when  $T_a$  or  $T_b$  is  $<8^\circ\text{C}$  or  $>34^\circ\text{C}$  as cold animals may cease ventilating and hot animals pant and breathe irregularly (Kayser, 1961; Oelkrug et al., 2013; Scholl, 1974; M.D.T., L.S., B.B. and F.v.B., personal observations). In the wild, the likelihood of tenrecs being exposed to a  $T_a$  outside of their tolerated range is rare. Thus, the highly plastic  $T_b$  exhibited by tenrecs may have worked well on an isolated island with moderate environmental temperatures and few homeothermic competitors. In more thermally variable environments, however, the plasticity seen in *T. ecaudatus* may not have allowed basoendothermic mammals to persist. Interestingly, the ranges of extant mammals like monotremes, marsupials, afrotherians and xenarthrans favor more moderate climates (Johansen, 1962).

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: M.D.T., F.v.B.; Methodology: M.D.T., J.R., J.R.B.L., F.v.B.; Software: J.R., J.R.B.L.; Formal analysis: M.D.T.; Investigation: M.D.T., L.S., B.B., A.K., A.J.M., T.R., A.R., C.F.R., D.S., J.F.S., C.S.R., E.S., J.R., J.R.B.L.; Data curation: M.D.T., D.S., J.F.S., C.S.R.; Writing - original draft: M.D.T., F.v.B.; Writing - review & editing: M.D.T., F.v.B.; Visualization: M.D.T., F.v.B.; Supervision: M.D.T., F.v.B.; Project administration: M.D.T., F.v.B.; Funding acquisition: F.v.B.

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