COPING WITH ENVIRONMENTAL FLUCTUATIONS: ECOLOGICAL AND EVOLUTIONARY PERSPECTIVES

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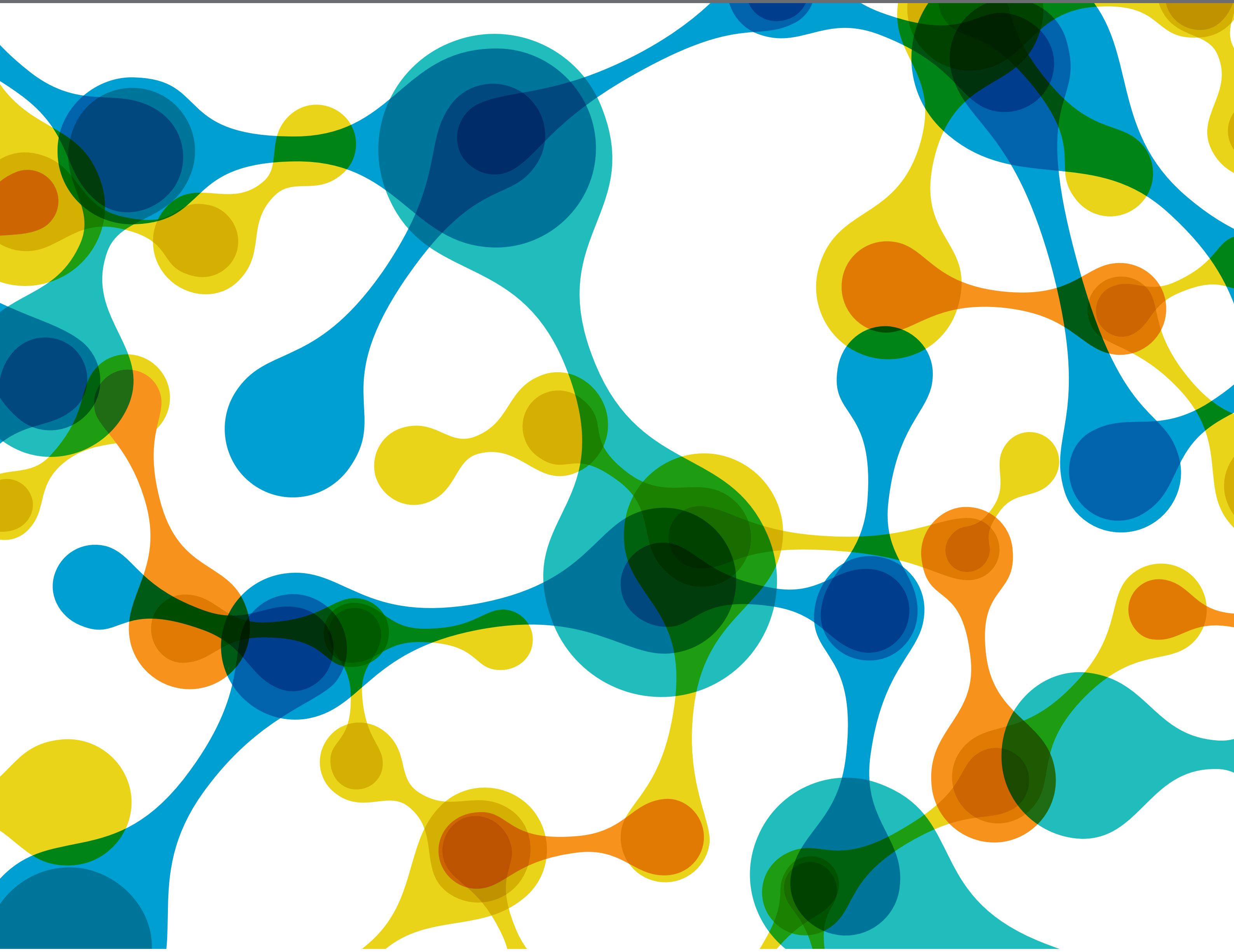

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COPING WITH ENVIRONMENTAL FLUCTUATIONS: ECOLOGICAL AND EVOLUTIONARY PERSPECTIVES

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Editorial: Coping With Environmental Fluctuations: Ecological and Evolutionary Perspectives

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Editorial of the Research Topic

Coping With Environmental Fluctuations: Ecological and Evolutionary Perspectives

Many organisms are adapted to endure substantial seasonal fluctuations in one or several environmental parameters. Recently, organisms across the globe also have to deal with accelerated climate change that is characterized by both a slow increase in mean temperature and increased frequency of extreme weather events (IPCC, 2013). To predict how this will affect wildlife, there is a need to understand how ecology and physiology have evolved to match the demands of an animal's current range. This Research Topic is comprised of both primary research and reviews that provide a comprehensive overview of recent advances in mechanistic and eco-evolutionary aspects of how animals cope with changing environments. Studies range over molecular, cellular, and organismal levels of enquiry and from individual animals to populations of mammals, birds, and selected ectotherms. Collectively, the Research Topic covers three main aspects of how animals respond to environmental fluctuations: (i) seasonal expression and evolution of hypometabolism and heterothermy; (ii) metabolic responses to climate change in dry, energy-limited, or hypoxic environments; and (iii) thermal sensitivity of individuals during development and its potential lifelong effects.

To face seasonal declines in resource abundance and/or temperature, many endothermic animals have developed the ability to save energy via heterothermy, whereby metabolic rate is substantially reduced and body temperature falls to within a few degrees of ambient (Ruf and Geiser, 2015). In his review, Geiser outlines the state-of-the-art of ecological, physiological, and life-history correlates of heterothermy. He remarks that hibernation (i.e., hypometabolic states lasting days or weeks), which occurs in mammals from all three subclasses but only in a single bird species, is not as seasonal as commonly thought and is strictly limited to winter in relatively few species. The expression of short bouts of daily torpor (i.e., hypometabolic states lasting a few hours) varies depending on environmental conditions and predation pressure. For this reason, the seasonal expression of torpor is not as clear-cut as commonly assumed and differs between true hibernators and daily heterotherms. Torpor is often associated with cold and seasonal habitats, but it is also common in the tropics and elsewhere at low latitudes. Nowack et al. report that the triggers of heterothermy and resulting patterns of metabolic rate and body temperature in low latitude heterotherms are often noticeably different from their high-latitude counterparts. Thus, while the underlying physiological regulation appears to be similar, low latitude species use torpor more flexibly, both in terms of seasonal timing and torpor bout length.

Dausmann et al. explore aspects of the regulation of heterothermy in a tropical species, the lesser hedgehog tenrec (*Echinops telfairi*) that uses torpor throughout the year. Tenrecs use variation in environmental temperature to both passively rewarm from torpor (hence saving energy) and as a *zeitgeber* to synchronize active and resting periods during summer and to time arousals during hibernation. Flexible year-round use of heterothermy is also used by the edible dormouse (*Glis glis*). The seasonal adaptations of this species are reviewed by Ruf and Bieber. The edible dormouse not only adapt to predictable seasonal variation in climate, but also to semi-predictable year-to-year variation in food availability, and day-to-day variation in weather. Edible dormice can hibernate for almost a year and skip reproduction in resource-poor years but breed intensively and show hyperphagia in years when food is bountiful. The switch between these extreme strategies is modulated by future reproductive potential with increased risk-taking behavior as the animals age.

The use of heterothermy can also differ between sexes. Noiret et al. report that physiological, metabolic and behavioral responses to food shortage are more flexible in females compared to males of the gray mouse lemur (*Microcebus murinus*). This reiterates the notion that heterothermic responses are closely adjusted to both environmental and state-dependent variables (Boyles et al., 2007; Geiser, 2013). Apart from a direct role in starvation avoidance, heterothermy may also impact survival through a reduction in extrinsic (predation) and intrinsic (somatic wear) mortality risk (Nowack et al., 2017). Constant et al. explore some aspects of the evolution of hibernation behavior by investigating how longevity is affected by phylogenetic signals and activity-time budgets across a range of mammalian hibernators. The authors show that longevity increases with the duration of the hibernation season, particularly in small (<1.5 kg) hibernators. On the other hand, Landes et al. propose that a flexible use of heterothermy may carry ecological and physiological costs, such as changes to the neuroendocrine system and increased free-radical production. While these costs may be marginal at each transition event, they could accumulate over time and therefore emerge with age. Nevertheless, it is assumed that the flexible use of torpor will be beneficial when species need to adapt to climate change.

Global change and the associated increase in extreme weather events pose an increasing threat to living organisms. This is clearly shown by Weyer et al. in their study of aardvarks (*Orycteropus afer*). Aardvarks are nocturnal and tightly control their circadian body temperature rhythm under normal conditions. A summer drought was associated with reduced foraging activity, increased mortality and relaxed body temperature regulation of aardvarks. Individuals that died progressively switched to almost complete diurnal activity. This does not bode well for aardvarks facing climate change, nor for the many animals that depend on aardvark burrows for refuge. Roussel and Voituron express similar concerns for the common toad (*Bufo bufo*). Their study found that mitochondria in this species produce ATP less efficiently and at a higher oxidative cost during an acute heat challenge. This might require extra resources to maintain ATP production and oxidative balance, leaving less energy for other tasks during heat stress. In contrast, Cooper et al. show that free-ranging zebra finches (*Taeniopygia guttata*) can accommodate heatwaves without major impacts on energy or water turnover, provided that drinking water is available. The birds avoid or limit activity during the hottest part of the day and showed pre-emptive feeding and drinking in preparation for inactive periods. It seems that cold weather could be more challenging than heat waves for this desert bird. Stochastic weather events alter not only food and water access, but also availability of other components linked to energy metabolism, such as dissolved oxygen in aquatic systems. Marshall and McQuaid show that snails (*Turritella terebra*) balance restricted oxygen availability in hypoxic seawater by downregulating metabolism and energy allocation to various physiological functions. Yet, compensation was incomplete since the snails incurred an oxygen debt in severe hypoxia.

Climate change and extreme weather events affect not only mature life forms, but can have pronounced effects during development when embryonic/fetal endotherms are actually poikilothermic and ectotherms typically cannot thermoregulate behaviorally. This can shape energy-based trade-offs, such as between thermoregulation and growth and (later) between survival and reproduction (Nord and Nilsson, 2016; Andreasson et al., 2018). Hence, this Research Topic also explores effects of environmental fluctuations during development. Since genetic adaptation is often slow, there is growing interest in how behavioral and physiological flexibility helps mitigate the challenges of climate change since flexible phenotypes allow organisms to optimize energy allocation when conditions change. In line with this, Nord and Giroud have reviewed recent findings on the effects of developmental temperature in birds and mammals and discuss when the early thermal environment is predisposing or constraining for lifelong thermoregulatory performance in fluctuating environments, and if such effects are heritable. Stawski and Geiser manipulated developmental temperature in yellow-footed antechinus (*Antechinus flavipes*) and measured the effects on adult metabolic rates. The study suggests considerable phenotypic plasticity in both sexes, which is likely vital for a short-lived species that only breeds once in a lifetime. While phenotypic plasticity in response to fluctuating environmental conditions during development is often advantageous, so-called counter-gradient variation (Levins, 1968) can oppose environmental influences to reduce the extent of phenotypic adaptation. Pettersen reviews such effects on genetic and phenotypic levels in reptiles, concluding that counter-gradient variation in developmental time may be an important adaptive response to decrease developmental costs for animals in cool climates. These contributions indicate the advantages of phenotypic plasticity in response to environmental conditions and support the notion that the pressure of climate change will likely select for increased phenotypic plasticity rather than for directional adjustment of phenotypes (Canale and Henry, 2010).

Collectively, this Research Topic summarizes new and important insights into understanding how ecological and physiological adaptation allows animals to thrive when environments fluctuate seasonally or annually. It also reveals potential implications in terms of life histories of these efficient, sometimes limited, adaptive responses of animal species. We hope that this Research Topic will provide a solid platform from which to embark on multidisciplinary research endeavors that will be required to understand the challenges and capacities for adaptation to climate change in the twenty-first century and beyond.

AUTHOR CONTRIBUTIONS

SG drafted the editorial. JN, AN, and KS made critical comments and substantially edited the manuscript. All co-authors approved the final version of the editorial and agreed to be responsible for all contents.

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ORIGINAL RESEARCH
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The Field Metabolic Rate, Water Turnover, and Feeding and Drinking Behavior of a Small Avian Desert Granivore During a Summer Heatwave

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Global environmental change is leading to an increase in the frequency, intensity, and duration of extreme weather events, so effective environmental management requires an understanding not only of the physiological response of organisms to increased mean temperatures, but also to extreme environmental conditions. To determine the physiological consequences of heatwaves on energy and water balance of arid-adapted zebra finches (*Taeniopygia guttata*), we measured field metabolic rate and water turnover rate of wild, free-living finches during a heatwave (consecutive days of maximum ambient temperature of 40–45°C) and during a cooler period (maximum ambient temperature of 28°C) during a summer drought. To understand how birds accommodated their energy and water requirements, we also monitored feeding and drinking behavior of zebra finches at the study site on hot and cold days over 2.5 months during the same summer. Zebra finches can accommodate heatwaves without major impacts on field energy or water turnover, even when the heatwave is superimposed on high summer temperatures and long-term drought, so long as drinking water is available. In fact, cooler periods may pose a greater energetic challenge than heatwaves during drought, when food availability is limited, due to the increased thermoregulatory cost of maintaining a high body temperature against a thermal gradient. Zebra finches avoided or limited activity during the most thermally challenging periods of the day. Their pre-emptive feeding and drinking in preparation for hours of relative inactivity at high ambient temperature, together with a high body water content and reduced midday activity and metabolic heat production, enabled zebra finches to maintain body mass during a heatwave. Predicting upcoming periods of unfavorably high ambient temperature, together with a high body water content, may be essential for survival by desert birds of extreme ambient temperature during heatwaves.

Keywords: bird, climate change, field metabolic rate, field water turnover rate, heatwave, foraging, temperature, zebra finch

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INTRODUCTION

Global environmental change is not only impacting average climatic conditions such as mean ambient temperature and annual rainfall but is also increasing the intensity, frequency, and duration of extreme weather events such as heatwaves (Meehl and Tebaldi, 2004; Tebaldi et al., 2006; Diffenbaugh and Field, 2013; IPCC, 2014). These extreme weather events may have a significant, immediate, and long-lasting impact on biodiversity, especially when combined with an underlying gradual mean change in climate (Saunders et al., 2011; Harris et al., 2018; Ruthrof et al., 2018). How animals respond to extreme environmental conditions provides exemplary insights into animal function as it is under these conditions that physiological processes must be most highly refined for survival. Therefore studying species during these extreme events will provide essential basic information about the physiological ecology of species. In addition, attempts to limit biodiversity loss by managing species in these changing conditions require a detailed mechanistic understanding of the physiological response of organisms to environmental variability (Harris et al., 2018; Conradie et al., 2019). However, our current understanding of the physiological function of most organisms is inadequate to determine effective mitigation strategies for even background climate change, and is even less able to predict and manage their response to unpredictable, extreme weather events (Chown et al., 2010; Ratnayake et al., 2019).

Birds are particularly susceptible to extreme weather events (Albright et al., 2017). They have a small body mass, are generally diurnally active and restricted to surface microhabitats, have high mass-specific energy and water requirements and a high body temperature (T_b) that is close to the lethal maximum for endotherms (Kendeigh, 1969; Withers et al., 2004; Williams and Tieleman, 2005). Mass mortality as a consequence of extreme heatwaves has been reported for various avian species, even iconic desert species such as budgerigars (*Melopsittacus undulatus*) and zebra finches (*Taeniopygia guttata*; Finlayson, 1932; McKechnie and Wolf, 2010; Saunders et al., 2011; McKechnie et al., 2012; Birdlife Australia, 2014), which inhabit naturally harsh, unpredictable arid environments. If these mortality events impact on uncommon or range-restricted species, or occur at a sufficiently large scale or frequency, they can have substantial impacts on the long-term viability of populations and even species (Saunders et al., 2011; Franklin et al., 2014; Ruthrof et al., 2018). For example, a sub-continental heatwave in Western Australia during 2011 was implicated in wide-spread abrupt and synchronized biotic effects in marine and terrestrial environments (Ruthrof et al., 2018), including reduced chick production and increased mortality of breeding little penguins (*Eudyptula minor*; Cannell et al., 2012). On a single day of extreme high temperature in southern Western Australia during 2010, at least 208 endangered Carnaby's cockatoos (*Calyptorhynchus latirostris*) were killed, with likely further deaths of orphaned chicks (Saunders et al., 2011).

During extreme heatwaves, terrestrial birds are impacted by the immediate trade-off between maintaining T_b at sub-lethal levels, typically <46°C (Kendeigh, 1969; Withers et al., 2004; Conradie et al., 2019; McKechnie and Wolf, 2019), using evaporative cooling, and fatal dehydration, generally assumed to be 11–15% body mass loss (Wolf, 2000; Albright et al., 2017). Birds can manipulate this trade-off by integration of physiological and behavioral responses, including reduction in activity and retreat to shaded, cooler microclimates, facultative hyperthermia, enhanced evaporative heat loss, and heat dissipation behaviors such as wing drooping and panting/gular fluttering (Wolf, 2000). However, these responses may affect a bird's ability to replenish body water by drinking, and may also hamper maintaining energy balance, due to reduced foraging opportunities and efficiency (Smit et al., 2016; Conradie et al., 2019; Funghi et al., 2019). For example, southern pied babblers (*Turdoides bicolor*) were unable to maintain body mass on days with maximum ambient temperature (T_a) >35.5°C due to the impact of heat dissipation behavior on foraging efficiency (du Plessis et al., 2012). Balancing energy and especially water budgets with fatal hyperthermia is especially challenging for small birds during periods of extreme heat compared to larger species, as they have a smaller body water pool and energy store and consequently a shorter survival window, coupled with higher rates of environmental heat gain and high mass-specific evaporative water loss (EWL) and energy use (Smit et al., 2016).

Here we examine how short periods of extreme temperature, superimposed over longer term conditions of drought and high summer temperature, impact on the energy and water turnover of wild, free-living zebra finches in an arid habitat in central Australia. Zebra finches are small (10–13 g), granivorous, estrildid finches that inhabit much of the Australian arid zone. They are a widely used model for investigating the physiological and behavioral mechanisms by which desert birds survive and reproduce in desert environments (Zann, 1996). We measure field metabolic rate (FMR) and water turnover rate (WTR) of free-living birds during periods of extremely high T_a (>40°C) when maximum T_a exceeds the average diurnal passerine T_b (McKechnie et al., 2012; Albright et al., 2017), and comparatively milder T_a (maximum $T_a \leq 28$°C). We compare these rates of energy and water turnover with diurnal patterns of foraging and drinking behavior, to provide information regarding the physiological and behavioral mechanisms that determine how extreme weather events may impact the survival and of small desert birds (McKechnie et al., 2012). We hypothesize that extreme heatwave conditions will reduce foraging opportunities, with a negative impact on energy turnover and significant body mass loss, and that water turnover and drinking rates will increase, during hot compared to cooler conditions.

METHODS

Zebra finches were studied at Gap Hills (31°05′ S, 142°42′ E), Fowlers Gap Arid Zone Research Station, approximately 112 km north of Broken Hill, New South Wales, Australia. The study occurred during December, January, and February 2018, within a 2-year period of extended drought and above-average temperatures (Australian Bureau of Meteorology, 2018). Four feeders, consisting of a 50 cm × 30 cm seed tray of commercial finch seed placed inside a 70 cm × 40 cm × 50 cm wire cage with a drop door, were opened at the site approximately 2 months before the study. The only free water known to be available within at least 5 km of the empty dam at Gap Hills was provided at two small drinking troughs (about 800 m and 100 m from the dry dam), filled from enclosed plastic water tanks with a float valve, and protected from large mammals and emus by a mesh fence. In the 50 months leading up to the fieldwork, a total of 771 finches had been captured at the site and were tagged subcutaneously with a passive integrated transponder (PIT; Minichip; Micro Products Australia, Perth, Australia), as part of other studies (e.g., Brandl et al., 2018; Funghi et al., 2019). All individuals were also banded with an individually numbered metal leg band (size 02) supplied by the Australian Bird and Bat Banding Scheme (ABBBS). Mark-recapture estimates suggest that approximately 350 individual birds were resident at the site during the study (Cooper, personal observation), but these were not all PIT tagged.

Field metabolic rate and WTR were estimated using the doubly labeled water method (Nagy, 1983; Speakman, 1997) to measure rates of CO_2 production and water flux for 10 wild, free-living zebra finches during a period of four consecutive days (18–21 January 2018) where maximum ambient temperature for the station exceeded 40°C (range 40–44.5°C; hot period), and for 10 finches during a period of 2 days (1–2 February 2018) when maximum ambient temperature was 27–27.6°C (cool period). The maximum temperature anomaly (deviation from the 30-year monthly average during 1961–1990; Australian Bureau of Meteorology) for these periods ranged from 4.4 to 8.3°C and −6.9 to −7.7°C respectively.

During the FMR and WTR measurements, only one feeder (and the two water tanks) remained open. A vehicle was used as a hide near the feeder, and finches were observed with binoculars and captured by the observer dropping the feeder door using a string trigger. Twenty finches were initially captured shortly after sunrise of the first day of the hot or cool period respectively. They were weighed to 0.1 g on an electronic balance, banded with both a uniquely numbered ABBBS metal band and a plastic color band, and given an IP injection of ~0.057 ml of water consisting of a 2:1 ratio of 98% enriched ^{18}O and ^{2}H; injection syringes were weighed before and after, to 0.0001 g. Finches were held in a cloth bag for approximately an hour (mean 1 h 9 min), after which an equilibration blood sample (~75 μl) was taken from the brachial vein before they were released. Ten finches in each experimental period were recaptured 18.7–71 h later by observing color-banded birds entering the feeder and triggering the drop door, and a recapture blood sample (~75 μl) was immediately taken. Blood samples (~75 μl) from six additional finches not involved in the turnover study were taken for analysis of background level of isotopes.

Blood samples were immediately flame-sealed on-site in the heparinized glass capillary tubes in which they were collected, using a butane burner. They were returned to the laboratory at the University of Western Australia, where water was extracted by vacuum distillation in flint glass pipettes. The enrichment of the injectate was calculated by diluting 0.061 g of the isotope solution into 7.56 g of water taken from the water troughs at the site. The solution was thoroughly mixed and samples before (background) and after (equilibration) addition of the injectate were also vacuum distilled and analyzed.

The stable isotope composition of distilled samples was analyzed by the West Australian Geochemistry Centre at the University of Western Australia (Skrzypek and Ford, 2014), using a Picarro L1115-I isotopic liquid water analyzer (Picarro, Santa Clara, California, USA). Prior to analyses, all samples were isotopically diluted with deionized water with a known isotope composition. The $\delta^{2}H$ and $\delta^{18}O$ raw values were normalized to the Vienna Standard Mean Ocean Water (VSMOW) scale after Skrzypek (2013), with three-point normalization replicated twice and calibrated against international standards provided by the International Atomic Energy Agency (Coplen, 1996). Body water content (BWC), water flux, and CO_2 production were calculated from these values (converted to ppm), after Speakman (1997). WTR was calculated as $r_{H2O} = N_H k_D$, where r_{H2O} is the H_2O turnover rate (mol h^{-1}), N_H is the average ^{2}H dilution space (mol), and k_D is the ^{2}H turnover (h^{-1}). FMR was calculated using Speakman's (1997) revision (i) of the Lifson and McClintock (1966) method as $r_{CO2} = N_O(0.48123k_O - 0.48743k_D)$, where r_{CO2} is the CO_2 turnover rate (mol h^{-1}), N_O is the average ^{18}O dilution space, and k_O is the ^{18}O turnover (h^{-1}).

The mean of the six background values was used in calculations for all birds. For five birds, for which equilibration samples could not be analyzed, the mean value for the equilibration samples of the other 15 birds was used. Total BWC was calculated from the ^{18}O dilution. FMR was converted from ml CO_2 day^{-1} to kJ day^{-1}, assuming 25 kJ L^{-1} CO_2 (Nagy et al., 1999). Metabolic water production (MWP) was calculated (based on a millet seed diet) as 0.62 mg ml^{-1} O_2 consumed (MacMillen and Hinds, 1983; MacMillen and Baudinette, 1993) and an oxyequivalence of 20.3 ml O_2 kJ^{-1} (Withers et al., 2016).

Feeding and drinking behavior was assessed from 1 December to 19 February (excluding the days on which FMR and WTR were measured) by monitoring birds accessing the feeders and water troughs using an 11-cm diameter antenna connected to a PIT tag reader (RFIDRW-E-232; Priority 1 Design, Melbourne, Australia) that recorded a tagged bird's unique ID code every time it passed through the antenna, with the date and time. Feeders were topped up with seed and water troughs checked daily, and the PIT recorder and timing checked (by passing a PIT through the antenna at a known time) and data downloaded every 1–5 days. Feeding behavior was analyzed for the four feeders on seven cool days when the maximum T_a was <30°C (mean maximum 25.6 ± 0.95°C, maximum range 22.2–29.8°C) and 17 hot days when the maximum T_a was >40°C (mean maximum 42.5 ± 0.42°C, maximum range 40.1–45.2°C), and for the water troughs for 26 hot and nine cool days, within 7 weeks of the FMR/WTR studies. The total number of times a bird passed through the antennae was summed for the four feeders and summarized into 30-min bin ranges, as were the data for the two water troughs.

Values are presented as mean ± S.E. with N = number of individuals, and times are Australian Central Summer Time (ACST; GMT + 10.5 h). Statistical analyses were accomplished with *statisti*XL (www.statistiXL.com). Body water content, FMR, and WTR were compared during hot and cool periods using two sample t-tests with a test, and if necessary correction, for equality of variance. Frequency of visits to the feeders and water troughs (analyzed separately) throughout the day was compared for hot and cool periods using a two-way contingency table (hot/cool vs. time of day). The frequency distribution of visits to feeders and water troughs during hot periods was directly compared to that during cool periods by using the distribution during cool periods as the expected values (adjusted to the same total number of visits during hot periods).

RESULTS

Birds captured during hot periods had a higher body mass (12.2 ± 0.20 g, N = 20) than during cool periods (11.5 ± 0.17 g, N = 20; t_{38} = 2.43; p = 0.020), but body mass did not differ significantly for those 10 individuals that were recaptured during each period and for which FMR and WTR were measured (12.1 ± 0.23 hot and 11.4 ± 0.21 cool, mean = 11.7 ± 0.17 g, N = 20; t_{18} = 2.09; p = 0.051). There was no difference in the body mass change between initial capture and recapture for hot and cool periods (mean = 0.06 ± 0.116 g; t_{18} = 0.966, p = 0.347).

Enrichment of ^{18}O and ^{2}H for recaptured birds was at least 268 and 216 ppm higher than the mean background value respectively, and exceeded the maximum measured for background samples by more than three (^{18}O) and seven (^{2}H) times. Body water content was 75.4 ± 2.14% (N = 20) and WTR was 4.5 ± 0.30 ml day^{-1} (N = 20), and neither differed between hot and cool periods ($t_{18} \leq 1.73$; $p \geq 0.187$). Field metabolic rate was significantly lower (t_{18} = 3.54, p = 0.002) during hot (29.4 ± 2.41 kJ day^{-1}) compared with cool periods (44.0 ± 3.35 kJ day^{-1}). As a consequence, MWP calculated from FMR was correspondingly lower during hot (0.953 ± 0.092 ml day^{-1}) compared to cool periods (1.317 ± 1.00 ml day^{-1}), and was 20 ± 2.3 and 34 ± 2.6% of the birds' WTR respectively.

There were 236 individual birds recorded visiting feeders, and 252 individuals recorded visiting water troughs during

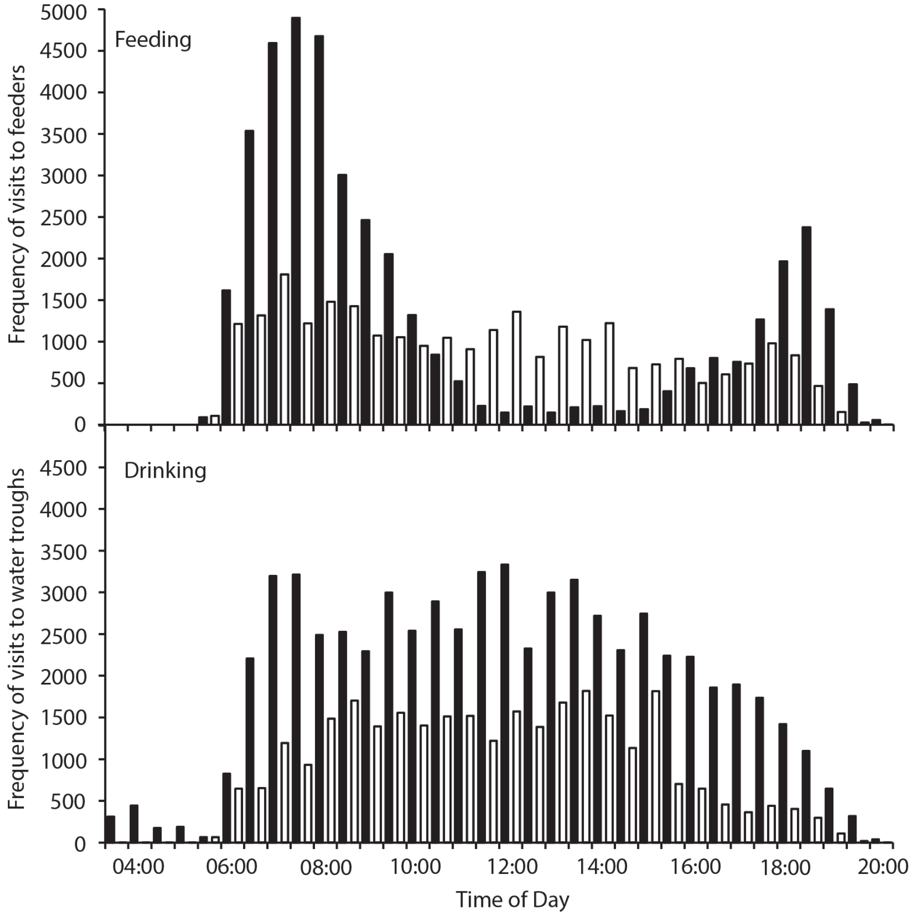

FIGURE 1 | Frequency of visits to four feeders (top panel) and two water tanks (bottom panel) by zebra finches (*Taeniopygia guttata*) in an arid habitat during hot periods (maximum daily T_a > 40°C; black bars) and cool periods (maximum daily T_a < 30°C; white bars).

the study period. Birds visited the feeders between 05:30 and 20:00, and the water tanks between 03:30 and 20:00, with between 707 and 7,838 total visits to the feeders, and 116–13,182 total visits to the water troughs recorded per day. Two-way contingency tables indicated a highly significant interaction between hourly visitation frequency and hot and cool periods for both feeding and drinking ($\chi^2_{29} = 13{,}310$, $p < 0.001$; $\chi^2_{33} = 2{,}817$, $p < 0.001$ respectively; **Figure 1**), and the frequency of feeding and drinking activity throughout the day was significantly different during hot (predominantly morning and evening activity) and cool (more uniform over the daytime) periods ($\chi^2_{33} = 38{,}433$, $p < 0.001$; $\chi^2_{33} = 172{,}814$, $p < 0.001$, respectively; **Figure 2**).

DISCUSSION

The wild, free-living zebra finches that we studied at Fowlers Gap are iconic desert birds, withstanding short periods of extreme temperature in their harsh, arid-zone environment. A period of 3–4 days with maximum $T_a > 40°C$ had minimal impact on the energy and water balance of the zebra finches. A combination of high BWC, a granivorous diet (with high digestibility that reduces foraging requirements), and the ability to behaviorally adjust foraging times in anticipation of high daytime environmental temperatures allows zebra finches to maintain body mass and meet their energy and water requirements when T_a exceeds thermoneutraltity for the majority of the day. Our data suggest that high T_a is actually less of an energetic stress than cooler days, and zebra finches are able to thermoregulate within tolerable limits without excessive water use, at least when maximum T_a does not exceed 45°C. Our results provide insight into the mechanisms by which birds can withstand extreme temperatures, and highlight situations when extreme environments may become lethal to birds.

Body Water Content

The BWC of birds is typically about 63–66% (Hughes et al., 1987; Ellis and Jehl, 1991; Speakman, 1997), although values of 70–80% have been reported, particularly for arid-habitat birds (e.g. Ambrose et al., 1996; Anava et al., 2000). Our values for zebra finches were relatively high (75%), and higher than for captive zebra finches (63%; Skadhauge and Bradshaw, 1974), and may indicate poor body condition with a low fat content (Johnson et al., 1985; Hughes et al., 1987; Ellis and Jehl, 1991). This presumably reflects the long-term drought and limited availability of food in an already harsh arid environment. This inference is supported by the observation that there had been little to no breeding activity of these birds in artificial next boxes (which generally support breeding, e.g., Griffith et al., 2008; Brandl et al., 2018) at the site during the previous year (Hurley and Griffith, personal observation). Although BWC can be overestimated if the period between injection of the isotope and sampling of blood for the equilibration sample is too long, our equilibration time of 1 h is typical for small birds (Speakman, 1997). A MWP of 1.317 ml day^{-1} (during cool periods when MWP was highest) would have produced only 0.055 ml of water during the 1-h equilibration period, which cannot account for the "extra" 1.028 ml of body water of a zebra finch with a calculated BWC of 75% compared

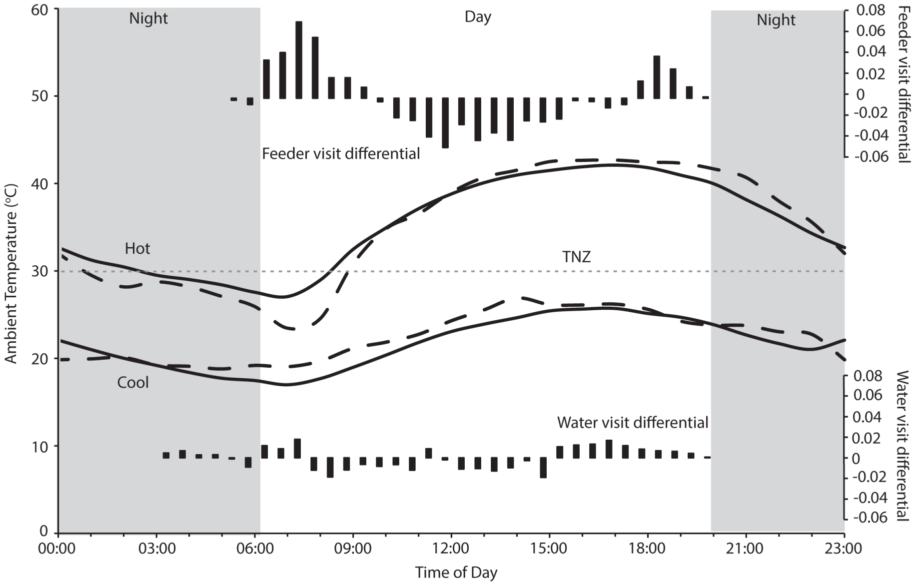

FIGURE 2 | Hourly temperature during hot and cool periods at Fowlers Gap research station, when visits to feeders and water troughs by zebra finches (*Taeniopygia guttata*) were monitored (solid lines), and when FMR and WTR were measured (dashed lines). The gray dashed line indicates thermoneutrality (TNZ; 30°C) for zebra finches (Calder, 1964; Cade et al., 1965) and the gray panels indicate the period from sunset to sunrise. Black bars indicate the differential of frequencies (from **Figure 1**) of visits on hot to cool days, standardized to total number of hot day visits, to four feeders (top panel) and two water tanks (bottom panel). Temperature data sourced from the Australian Bureau of Meteorology for Fowlers Gap (station number 046128).

to 66%. Body water content can also be overestimated if the injection volume was overestimated, for example by leakage from the injection site, or loss of injection solution from the syringe after pre-injection weighing. We cannot unequivocally eliminate this as a source of error, but did not observe leakage or spillage of injection solution. We therefore consider that our high value for BWC of zebra finches is real, and likely adaptive (see below).

Field Metabolic Rate and Water Turnover Rate

Our measures of FMR and WTR are consistent with allometric predictions for desert birds, and generally consistent with observations from captive individuals. Field WTRs of our zebra finches (4.5 ml day^{-1}) were 99–113% of that predicted for desert birds, and 49–57% of that for all birds, after Tieleman and Williams (2000), and 80–92% and 74–85% respectively after Nagy and Peterson (1988), reflecting the more frugal water turnover of birds in arid habitats. The WTR we measured for wild, free-living zebra finches was also within the range of water intake rates of 3.8–5.4 ml water day^{-1} measured for captive zebra finches (Skadhauge and Bradshaw, 1974). The small body size and therefore relatively high metabolic rate of small birds means that they can meet a considerable proportion of their daily water requirements *via* MWP (Bartholomew, 1972; Bradshaw, 2003). For wild, free-living finches in an arid habitat in summer, MWP accounted for 20–34% of WTR, which was consistent with the 31.3% of daily water flux of zebra finches in the laboratory (Skadhauge and Bradshaw, 1974).

The extremely hot conditions during our FMR measurements did not negatively impact the zebra finches' energy use, with finches maintaining body mass over the hot period. We calculated 1.9–2.9 g fresh matter intake day^{-1} for our finches (from FMR for a granivore after Nagy, 2001), which is 63–90% of that predicted for a passerine bird and 32–46% of that predicted for a desert bird, reflecting the high metabolizable energy content, and therefore low fresh matter intake, of a granivorous diet (Nagy, 2001). The FMR that we measured for our birds during this study was 2.0–2.8 times BMR for this species (Rønning et al., 2005; Cooper et al., in review), consistent with the generalization that FMR is 2–3 times BMR (Degen and Kam, 1995; but see Koteja, 1991 and Ricklefs et al., 1996).

Feeding and Drinking

On days of extremely high T_a, zebra finches dramatically reduced foraging, and to a lesser extent drinking, during the hottest part of the day, as is typical for birds experiencing extreme heat and solar radiation (Wolf, 2000). Activity for foraging and drinking will increase metabolic heat production, and doing so in unshaded locations typical of arid environments also exacerbates an already high T_a with high incident solar radiation (Abdu et al., 2018). This may increase the effective T_a experienced by a small bird by about 12°C (Wolf, 2000), meaning birds in our study would be exposed to temperatures in excess of 57°C, which is well above the tolerance limit of most passerine birds (Abdu et al., 2018; McKechnie and Wolf, 2019). Therefore, zebra finches, like other desert birds, presumably face a trade-off between maintaining energy and water balance during extremely hot conditions and accessing food and water with exacerbated heat gain (Smit et al., 2016).

We found that zebra finches reduced their foraging activity for approximately 8 h in the middle of the day on hot compared to cool days. Finches balanced the reduced midday foraging on days with a high maximum T_a with increased foraging in the early cooler part of the day, and to a lesser extent in the evening (**Figure 1**). In contrast, birds foraged more consistently throughout the day during cooler weather. Previous studies of zebra finches at this site found that increased evening, rather than morning, foraging compensated for reduced midday foraging (Funghi et al., 2019), but for diurnal birds this strategy relies on sufficient environmental cooling in the evenings before dark for birds to forage. Environmental conditions are cooler in the early mornings than evenings (**Figure 2**), so anticipatory early morning foraging is likely a more favorable strategy during extremely hot conditions. However, birds must be able to anticipate reduced midday foraging to increase feeding in preparation for prohibitive daytime and evening conditions. Our data suggest that zebra finches were able to anticipate hot midday temperatures, presumably from overnight and early morning conditions, and increase their morning foraging accordingly. Similar pre-emptive responses to high maximum daytime T_a have been described for red kangaroos (*Osphranter rufus*) and western gray kangaroos (*Macropus fuliginosus*), whose nychthemeral reductions in T_b were greater in the early mornings of days with high maximum temperature than on days with lower maximum temperature, presumably facilitating heat storage later in the day (Brown and Dawson, 1977; Maloney et al., 2004). However, unlike kangaroos, which appear to pre-empt a favorable thermal response *via* an autonomous physiological feedback mechanism to environmental conditions (warming of the skin surface, resulting in distribution of core body heat to the cooler periphery and therefore a decrease in core T_b; Maloney et al., 2004), our data suggest that finches behaviorally "plan" to withstand upcoming harsh environmental conditions.

Mass, Energy, and Heat Balance

We found no difference in the zebra finches' ability to maintain body mass over consecutive hot or cool days, despite evidence that reductions in foraging time and/or efficiency due to trade-offs with thermoregulatory behavior may significantly impact on energy balance of other birds, and that birds at high T_a may not be able to maintain body mass after consecutive warm days (du Plessis et al., 2012; Cunningham et al., 2015; Van de Ven, 2017). In fact, zebra finches maintained a higher body mass when it was hotter, and T_a was within or above the thermoneutral zone for the majority of each day (**Figure 2**). Although total foraging time of zebra finches during hot weather is less than during cool weather (Funghi et al., 2019), the reduced requirement for MHP means that total energetic requirements are lower, and a high-energy, highly digestible granivorous diet (MacMillen, 1990; Nagy, 2001) minimizes the fresh matter intake required for these birds compared to other species. Our data suggest that extremely hot weather did not place energetic constraints on the zebra finches by limiting feeding. Rather, cooler periods posed greater energetic challenges by requiring increased MHP to maintain a homeothermic T_b at T_a below thermoneutrality (**Figure 2**), and this may have been more challenging during a drought when natural food availability is low. Body size, diet, and foraging strategy presumably influence which bird species are most likely to experience energetic constraints from high temperatures (Edwards et al., 2015; Smit et al., 2016). Granivores, even those with food involving considerable handling time, may be able to meet their daily energetic requirements with "time to spare" and therefore do not need to forage for the entire daytime period. For example, cockatoos can meet their energetic returns by foraging for only 4–5 h a day (Cooper et al., 2002) and rest in shady locations for many hours, especially during summer (Noske, 1982; Cooper, personal observation). The finches in this study were provided with supplementary food to facilitate capture and data collection, which presumably assisted them in meeting their energy requirements more quickly than if they had to forage widely for all of their food. However, foraging behavior of individual birds suggests that they maintained natural foraging patterns when visiting the feeders (Funghi et al., 2019). A high BWC (75 vs. ~66%) and lack of breeding at the site also suggest poor body condition, and are evidence that the supplemental food provided was not excessive and did not compensate entirely for a scarcity of food as a consequence of the long-term drought.

Due to lower FMRs during hot periods, MWP was a smaller proportion of the total WTR of zebra finches, and therefore this difference (14% of WTR) had to be gained, presumably by increased drinking. Indeed, finches drank more frequently on hot compared to cool days (**Figure 1**), which maintained the same WTR. Measurements of physiological variables for zebra finches in the laboratory (Cooper et al,. in review) suggest that, despite their small body size, they use facultative hyperthermia to conserve water over the short term; a water savings of 9.17 mg g^{-1} h^{-1} measured for hyperthermic finches at 40°C (Cooper et al., in review) equates to a water savings of 57% of the mean water use by wild birds over 8 h. Finches can therefore delay dissipation of body heat until cooler environmental conditions facilitate non-evaporative heat loss, and this presumably is why birds during hot periods did not have a higher overall WTR than those during cool periods.

Despite overall higher drinking rates, distribution of visits by finches to water troughs decreased more in the middle of the day when it was hottest, and they increased their visits to water more in the early mornings and especially late afternoons (**Figure 2**), presumably preparing for, and then replenishing, water lost throughout the day. As they did for foraging, birds appear to be anticipating upcoming limitations on daytime drinking by drinking earlier, and more frequently early in the day, on extremely hot days. A high BWC may reduce the impact of short-term dehydration (e.g., as for amphibians; Hillman et al., 2009), and therefore limit reliance on visits to water during the heat of the day. If we assume that an 11.7-g bird with 66% BWC (Speakman, 1997) can lose ~11% of body mass as water before facing lethal dehydration (Wolf, 2000), then it can lose 1.287 ml of water (29% of their WTR) to retain 6.4 ml of body water. However, a bird of the same body mass starting with 75% body water can lose 2.34 ml of water to retain 6.4 ml of body water; they can lose 54% of their daily water turnover before risking lethal dehydration, extending the period birds can avoid drinking during periods of extreme environmental temperature. Indeed, finches did increase their drinking in the late afternoon on hot compared to cool days, presumably replenishing their body water store once the contribution of solar radiation to environmental temperature had decreased (**Figure 2**). Spinifexbirds (*Eremiornis carteri*; Ambrose et al., 1996) and Arabian babblers (*Turdoides squamiceps;* Anava et al., 2000) are other arid-habitat birds that have similarly high BWCs (up to 80%) that may also serve as a water store.

Implications for Understanding High-Temperature Avian Mass Mortality

Overall, our results indicate that heatwaves of 40–45°C maximum T_a can be accommodated by arid-adapted zebra finches without major impacts on energy and water turnover. Finches modified their behavior to avoid or limit activity during the most thermally challenging periods of the day and, together with a high BWC that presumably increased their body water store, were able to maintain body mass during a heatwave by pre-emptively feeding and drinking in preparation for hours of relative inactivity at high T_a. We can apply this information to identify potential situations where avian mass mortality events can occur. Predicting upcoming periods of unfavorably high T_a may be essential for survival of extreme T_a. One recently recorded avian mortality event, at Hopetoun, Western Australia, occurred when an extremely hot day (maximum T_a = 48°C) occurred between two much cooler days (maximum T_a = 24–26°C; Saunders et al., 2011; **Figure 3**), and following at least a fortnight of relatively cool summer maximum temperatures. Early morning temperatures on this day were indistinguishable from those of the preceding days; so, it is likely that birds experiencing this hot day did not "prepare" sufficiently in the cooler morning when feeding and more importantly drinking were possible, if the extremely high T_a was unexpected. The short-term predictability of heatwaves may be a determinate of avian survival. Another recent avian mass mortality event occurred at Overlander Roadhouse in the mid-west region of Western Australia (Birdlife Australia, 2014). In this case, maximum temperatures were very extreme, >40° for more than a fortnight, and ≥45°C for several consecutive days (**Figure 4**). Unfortunately, hourly temperature data are not available for a weather station close to this site, but it is likely that temperatures did not drop sufficiently during daylight hours over this prolonged heatwave to allow sufficient feeding or drinking to maintain energy and water stores. In addition, a previously good season with high winter and spring rainfall that facilitated breeding and subsequent large numbers of birds in the region (Birdlife Australia, 2014)

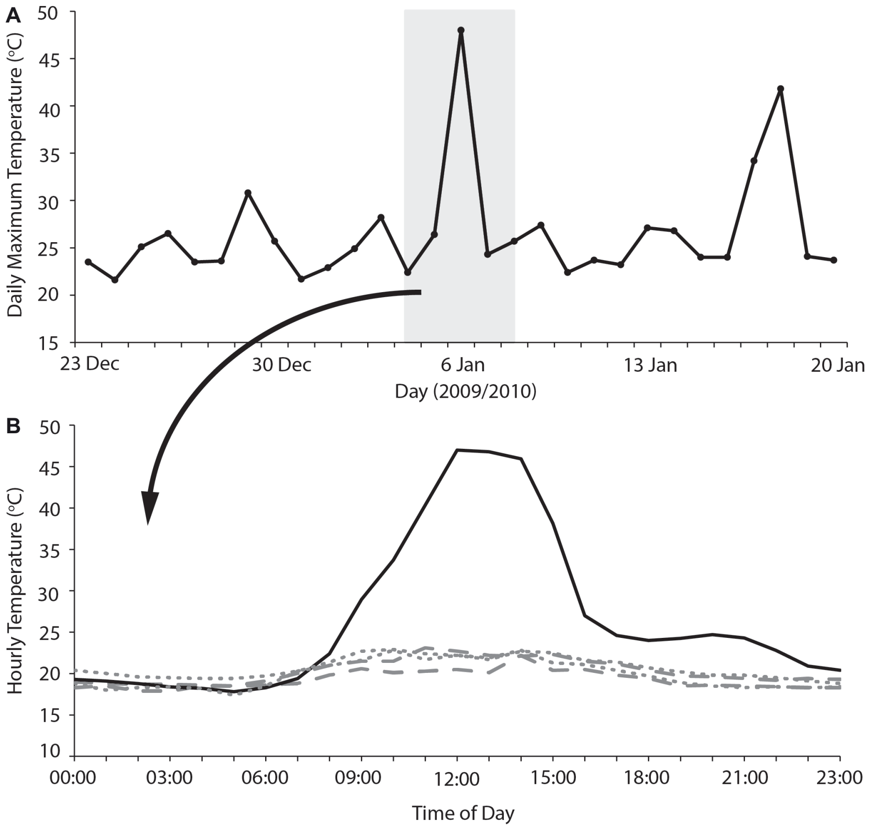

FIGURE 3 | (A) Maximum daily temperatures for 2 weeks either side of an avian mass mortality event that occurred on the 6/2/2010 at Hopetoun, Western Australia. The gray shaded section indicates the 5 days where hourly temperature is plotted **(B)** for the 2 days before (dashes), 2 days after (dots), and the day of the avian mass mortality event (solid). Temperature data sourced from the Australian Bureau of Meteorology for Hopetoun North (station number 009961).

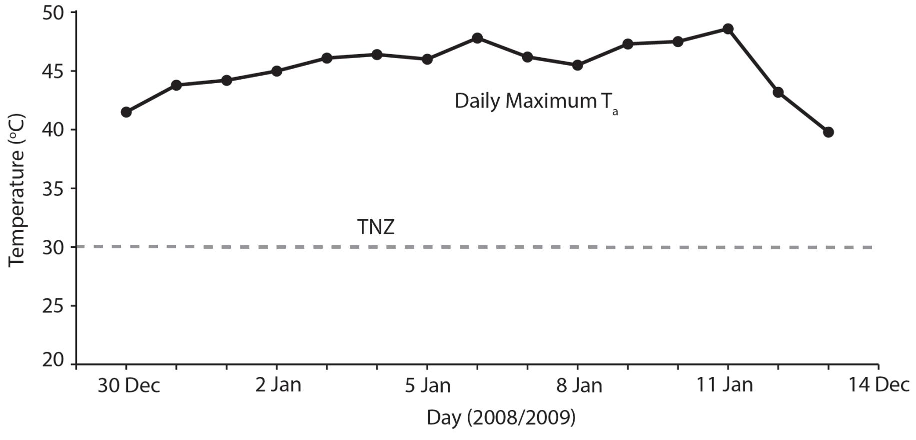

FIGURE 4 | Maximum daily temperatures (black symbols) for 2 weeks during which time an avian mass mortality event occurred during January 2019, in the Pilbara region of Western Australia. The gray dashed line indicates thermoneutrality (TNZ; 30°C) for zebra finches (Calder, 1964; Cade et al., 1965). Temperature data sourced from the Australian Bureau of Meteorology for Gascoyne Junction (station number 006022).

presumably meant that birds were in good body condition, and may not have had the high lean BWC that we hypothesize may be critical for facilitating dehydration tolerance during the heat of the day.

To manage species and minimize biodiversity loss under the inevitable climatic changes that are occurring as a consequence of anthropogenic activity, we need a greater mechanistic understanding of how species physiologically accommodate environmental variability (Harris et al., 2018; Conradie et al., 2019). Our results reveal the consequences of short periods of high temperatures for an iconic desert bird in its natural habitat, and indicate the physiological and behavioral mechanisms by which zebra finches withstand extreme environmental conditions. This information has revealed that periods of low T_a during summer and particularly during droughts may pose greater energetic challenges than heatwaves for granivores. We also identify high BWC and predictable foraging limitations as potentially important elements for avian survival during periods of extreme heat. This information may assist in predicting and managing avian responses to periodic extreme weather events.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Macquarie University and Curtin University Animal Ethics Committees (ARA 2017/024 and ARE2017-16).

AUTHOR CONTRIBUTIONS

CC and SG designed the study, provided equipment and logistical support, and obtained funding. CC and LH conducted the fieldwork. CC and PW carried out laboratory procedures and analyzed the data. CC drafted the manuscript. All authors edited the manuscript.

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Growing Up in a Changing Climate: How Temperature Affects the Development of Morphological, Behavioral and Physiological Traits of a Marsupial Mammal

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Climate change is likely to affect many mammalian phenotypes, yet little is known whether and how phenotypic plasticity is involved in responding to thermal challenges during mammalian development. We investigated the effect of continuous cold or warm exposure during development on morphological, behavioral, and functional variables of yellow-footed antechinus (*Antechinus flavipes*), a semelparous Australian marsupial mammal. Captive-bred young were exposed to two ambient temperatures (T_a), cold (17°C) or warm (25°C), once weaned. Treatments were reversed and metabolic rate (MR) measurements repeated after 2 months. We measured body mass weekly, activity continuously, and MRs over a range of T_a once they were adults. Growth rate was similar in both groups, but was faster in males. Antechinus in the warm group were initially more active than the cold group and decreased activity when exposed to cold, whereas the cold group increased activity when exposed to warm. Interestingly, females changed their night-time activity when T_a was changed, whereas males changed their daytime activity. MRs were originally lower in the warm group in comparison to the cold group for both sexes and increased slightly for females, but not for males, after being exposed to cold. After exposure to warm T_a, the MRs of the cold group decreased significantly over the entire T_a-range for both sexes. Our results reveal that temperatures experienced during development can influence behavioral and physiological traits in antechinus. Such phenotypic plasticity is vital for a species that within 1 year is dependent on a single breeding event and experiences a complete population turnover.

Keywords: activity, antechinus, body mass, endotherm, metabolic rate, phenotypic plasticity

INTRODUCTION

Ambient temperature (T_a) is known to influence critical developmental stages of many organisms. Traits affected by T_a during development range from body size to sex to energetics and, importantly, adult phenotypes are a result of the environment in which the individual developed (Scharloo, 1989; Pigliucci et al., 1996). An increase in mean T_a due to climate change is likely to substantially affect organism development. However, little is known about how species respond morphologically, behaviorally and physiologically during development to new thermal challenges and such data are particularly lacking for endotherms (Williams et al., 2008; Huey et al., 2012).

Temperature effects on growth rates in ectotherms are relatively well understood. In general, most ectotherms grow more slowly under cold conditions in comparison to warm conditions (Angilletta, 2004). Conversely, how different T_a affect growth rates during development in endotherms is poorly understood, although for many organisms cold environments can often select for larger body sizes, known as Bergmann's rule (Bergmann, 1847; Meiri and Dayan, 2003; Angilletta, 2004). Growth rate is vital in species that have a short period in which to become mature in order to either reproduce before perishing or to survive their first winter period. For example, species that inhabit extreme arctic and alpine environments have a protracted active season in which to gather enough fat reserves or food to survive the long winter (Morrison et al., 1954; Bieber et al., 2017). Importantly, T_a has an immediate effect on the activity patterns of many endotherms. Most notably, many species of birds and mammals reduce activity levels as T_a decreases (Vickery and Bider, 1981; Brice et al., 2002; Ashdown and McKechnie, 2008), because remaining active is energetically costly at colder T_a. Therefore, activity patterns in many endotherms change seasonally, such that they display more activity during the warmer summer months. It may be that the activity level of an individual during development as a result of the T_a can impact not only on their adult activity levels, but also on their resulting growth rates and metabolic rates (MRs).

Understanding an animal's MR is vital to interpreting how the individual functions in its current environment and how it may adapt to new conditions. In particular, MR governs both day-to-day energetic requirements and more long-term variables such as growth rate and survival (Anderson and Jetz, 2005; Burton et al., 2011). Further, mechanisms that are employed to balance energy loss and gain on a daily basis must be under strong selection pressure, as being in a negative energy state is highly undesirable (Auer et al., 2016; Stawski et al., 2017a). In order to maintain a high and stable body temperature (T_b), endotherms have much higher and adjustable MR in comparison to ectotherms. This allows endotherms to be active largely independent of environmental T_a. However, both cold and hot T_a increase MR in order to produce heat or remove heat, respectively. This relationship of MR with T_a is best represented by the thermoregulatory curve, which also encompasses the thermoneutral zone (TNZ). The TNZ is the range of T_a where the animal does not need to thermoregulate and can display basal metabolic rates (BMR). Below the TNZ, the MR must increase to compensate for heat loss, above the TNZ it increases to facilitate heat loss via evaporative cooling. The thermoregulatory curve provides a useful tool to determine how environmental variables affect the energetics of endothermic animals when at rest. For example, many endotherms display a seasonal shift of the thermoregulatory curve in order to acclimate to varying environmental conditions, such as a change in weather and photoperiod, throughout the year (Zhao et al., 2010; Bozinovic et al., 2011; Boratyński et al., 2016). However, even more advantageous than flexibility in response to predictable seasonal changes, is phenotypic flexibility in response to unpredictable changes (Riek and Geiser, 2012; Boratyński et al., 2016). which would be advantageous to adapt to climatic changes.

The aim of our study is to provide quantitative data on the effect of continuous cold and warm exposure during development on morphological and functional variables of the marsupial antechinus. These insectivorous marsupials have a most unusual life history. In the wild they breed once a year over a short 2-week period (Naylor et al., 2008). The mating period is followed by a complete male die-off at about 11 months of age, attributed to a combination of stress-related factors (Naylor et al., 2008). Females, on the other hand, must survive at least for 15 months until their large litters of young are weaned and a few females may live for a second year (Naylor et al., 2008; Rojas et al., 2014). Importantly, apart from these females, there is only a single cohort of individuals at any given time. This type of life history pattern, known as semelparity, is rare in mammals and typically only in those that inhabit predictable seasonal environments as their mating period usually commences based on changes in photoperiod (Naylor et al., 2008). Because antechinus exhibit a complete population turnover in a single year they offer an ideal model for investigating effects of T_a on morphological and functional variables to gain a better understanding on how animal populations are likely to respond in the short term to thermal challenges they will experience during climate change. We hypothesized that cold-rearing and warm-rearing will affect morphology, behavior and physiology irreversibly. Importantly, changes in activity and MR are likely to be correlated in order to cope with the varying energetic demands created by the different thermal treatments. If observed, such phenotypic responses are important for coping with different climates over a wide range of distribution, but also play a crucial role for enhancing survival during climate change.

MATERIALS AND METHODS

All procedures were approved by the University of New England Animal Ethics committee.

Initially, eight yellow-footed antechinus (*Antechinus flavipes,* Marsupialia, Dasyuridae; five females, three males) were captured in the wild from Aberbaldie Nature Reserve (31°04′24″S, 151°25′34″E) in Australia and studied in a different experiment (see Stawski et al., 2017b). After the conclusion of this previous experiment (end of July) these wild-caught adults were held together in a large outdoor aviary with access to several nest boxes placed at various heights. They were supplied with *ad libitum* food and water. From July to October the reproductive status of these adults was monitored and to ensure successful reproduction, in mid-August all individuals were transferred to an indoor facility and kept in individual cages (see Stawski and Rojas, 2016). The females gave birth to a total of 19 young (9 females, 10 males) from early to mid-September. Once the young were weaned [early December at ∼ 100 days of age (see Westman et al., 2002)], they were separated from their mothers and placed into individual cages (week 0 on **Figure 1**). These juveniles were then randomly split into two treatment groups, warm or cold, and

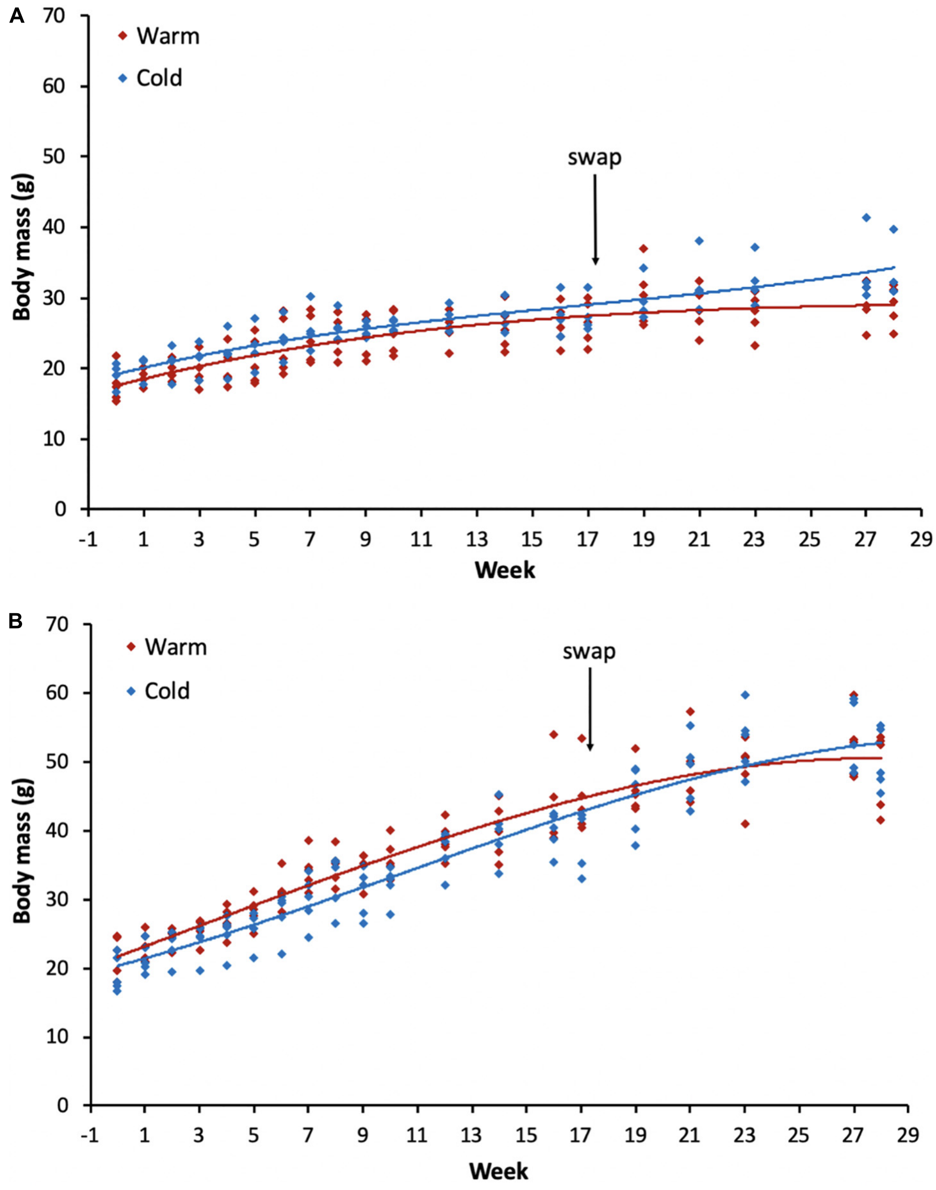

FIGURE 1 | Body mass changes for all individuals post-weaning (from ∼100 days old) throughout the experiment for **(A)** females and **(B)** males for each of the treatment groups. The arrow indicates the week when the acclimation temperatures were reversed for the groups. Red lines indicate the group that was initially placed in the warm treatment and the blue lines the group in the cold treatment. Week 0 represents body mass on the day the individuals were placed into the temperature controlled rooms.

placed into rooms at a T_a of 16.7 ± 1.3°C (n = 199 days; N = 4119 measurements; cold) or 24.7 ± 1.3°C (n = 199 days; N = 4119 measurements; warm). Body mass (± 0.1 g) of all individuals was measured with an electronic balance once a week throughout the duration of the experiment.

Activity patterns of all of the juvenile antechinus was measured throughout the experiment with passive infrared sensors attached to individual cage lids and recorded with custom-made data loggers (Körtner and Geiser, 1995). The sensors pick up movements and these were summed on the data loggers over 10-min periods. Sensors were placed facing directly down into the cage of the animal, which prevented the sensors from picking up any movement from animals in nearby cages. Short time periods (∼5 min) when experimenters were feeding and replacing water in the cages or longer time periods (∼60 min) when animals were being weighed and the cages cleaned, were noted and these data were removed from analyses.

When the juvenile antechinus reached adult size (end of March at ∼220 days of age), the thermal response of MR as a function of T_a was quantified for each individual. MR was measured as rate of oxygen consumption ($\dot{V}O_2$) and was obtained via open-flow respirometry. A linear mass loss throughout measurements was obtained by weighing the antechinus before and after each measurement and this was used for calculation of mass-specific $\dot{V}O_2$ values. A FOX analyser and a FC-1B analyser (both from Sable Systems International, Las Vegas, NV, United States, resolution 0.001%) were used to obtain the $\dot{V}O_2$ measurements. The FOX analyser system switched in sequence between three animal channels and a reference channel every 3 min, whereas the FC-1B analyser system switched between two animal channels and a reference channel every 3 min. Mean $\dot{V}O_2$ values were derived from periods when oxygen consumption was minimal and stable for >3 consecutive readings (i.e., over > 27 min). Rotameters (Aalborg, NY, United States) were used to control the rate of airflow into the chambers and mass flow meters (Omega FMA-5606, Stamford, CT, United States) were used to measure the rate of airflow. A flow rate of 500 ml O_2 min^{-1} was used for each animal chamber and 200 ml O_2 min^{-1} for the analyser subsample. Voltage outputs from flow meters and oxygen analysers were recorded on a computer via a 14-bit A/D converter card. Air samples were dried using silica gel. Glass jars (1000 ml) were used as respirometry chambers to house the antechinus during measurements. Once the antechinus were in these chambers they were placed within a temperature-controlled unit. T_a within the temperature-controlled unit and in each of the individual chambers was measured at the same time as $\dot{V}O_2$ readings by a calibrated thermocouple (±0.1°C, Omega DP116 digital thermocouple thermometer, Stamford, United States); these data were transferred to the computer via the A/D converter card. $\dot{V}O_2$ was calculated by using equation 3a of Withers (Withers, 1977) and G. Körtner wrote the data acquisition program.

Two experimental protocols for the MR measurements were used. For both protocols the animals were measured during their daytime resting period to ensure resting MR was obtained. For the first protocol the animals were placed into the chambers at T_a 18°C and the T_a was increased in 4°C increments every 2 h up to 30°C. The T_a began at 14°C for the second protocol and was reduced to 10°C after 3 h. The exact T_a of each chamber varied; therefore the individual chamber T_a were used when analyzing the data.

At the end of the first round of MR measurements acclimation T_a were reversed (at ∼230 days of age). After the reversal, weekly body mass measurements were continued and activity was again measured continuously with the passive infrared sensors. After 3 months of acclimation (at ∼320 days of age) the MR measurements were repeated as described above. This was to ensure that measured functional variables did not simply reflect temperature acclimation rather than developmental phenotypic adjustments.

The two treatment groups (cold and warm) were split into the following four groups for analysis to account for the reversal treatment:

- Cold: individuals that were initially housed under cold conditions.
- Cold-Warm: individuals that were initially housed under cold conditions and then transferred to warm conditions.
- Warm: individuals that were initially housed under warm conditions.
- Warm-Cold: individuals that were initially housed under warm conditions and then transferred to cold conditions.

All data analyses were conducted using “R” Studio (R Development Core Team, 2009). To test what factors (date, body mass, sex, treatment, T_a) affected the measured variables (body mass, whole day activity, daytime activity, night activity, resting MR, basal metabolic rate) linear mixed effects models were fitted (function “lmer” in package “lme4”) and ranked using AIC (function “dredge” in package “MuMIn”). To account for repeated measures, individuals were included as a random effect. Once the top model had been selected for each of the measured variables, a Shapiro test was run on the residuals to ensure normal distribution, which was the case for all of the variables. Afterward, a Tukey test (function “lsmeans” in package “lsmeans”) was run to see what treatment groups differed significantly from each other ($p < 0.05$).

RESULTS

Growth

The best-fit model revealed that growth rates among sexes and treatment groups were described by date, sex, treatment and an interaction between sex and treatment (**Table 1**). As expected, individuals increased body mass with time throughout the experiment across both sexes and all treatment groups (**Figure 1**). No significant differences were found between the treatment groups for the same sex (p-values ranged from 0.100 to 1.000). However, there was a significant difference between sexes (p-values ranged from <0.0001 to 0.035), such that males were typically heavier than females. Starting body mass for females in the warm treatment was 17.7 ± 2.2 g ($n = 5$) and in the cold treatment was 19.1 ± 1.5 g ($n = 4$), whereas for males was 21.9 ± 2.7 g ($n = 5$) in the warm treatment and 19.2 ± 2.4 g ($n = 5$) in the cold treatment. The final body mass recorded during the entire experiment for females in the warm-cold treatment was 28.9 ± 2.6 g ($n = 5$) and in the cold-warm treatment was 33.5 ± 3.7 g ($n = 4$), whereas for males was 48.9 ± 5.2 g ($n = 5$) in the warm-cold treatment and 50.3 ± 4.0 g ($n = 5$) in the cold-warm treatment.

Activity

For all of the activity variables (whole day, day, night) the top model showed that they were influenced by body mass, sex, treatment and the interactions between these variables (**Table 1**). For both sexes and all treatment groups, activity levels increased as body mass increased.

No significant differences were found in whole day activity levels between the sexes and also between treatment groups (**Figure 2**; p-values ranged from 0.100 to 1.000). The only significant differences for whole day activity were found for females and males after individuals had been transferred to the new treatment conditions. Both males and females increased activity when transferred from the cold treatment to the warm

TABLE 1 | The four best-fit models for each of the measured variables.

Variable	Model	AICc	Delta
BM	**date + sex + treatment + sex:treatment**	**12797.9**	**0.0**
	date + sex + treatment	15214.0	2416.1
	date + treatment	15239.5	2441.5
	date + sex	15257.9	2459.9
WDA	**bm + sex + treatment + bm:sex + bm:treatment + sex:treatment**	**52961.3**	**0.0**
	bm + sex + treatment + bm:treatment + sex:treatment	53004.3	42.9
	bm + sex + treatment + bm:sex + bm:treatment	53045.9	84.7
	bm + sex + treatment + bm:sex + sex:treatment	53094.5	133.2
DA	**bm + sex + treatment + bm:sex + bm:treatment + sex:treatment**	**49834.4**	**0.0**
	bm + sex + treatment + bm:treatment + sex:treatment	49884.9	50.5
	bm + sex + treatment + bm:sex + sex:treatment	49980.4	146.0
	bm + sex + treatment + bm:sex + bm:treatment	50017.2	182.8
NA	**bm + sex + treatment + bm:sex + bm:treatment + sex:treatment**	**51251.9**	**0.0**
	bm + sex + treatment + bm:treatment + sex:treatment	51292.8	40.8
	bm + sex + treatment + bm:sex + sex:treatment	51300.3	48.4
	bm + sex + treatment + sex:treatment	51334.8	82.9
RMR	**sex + T_a + treatment + sex:T_a + sex:treatment + T_a:treatment**	**−99.2**	**0.0**
	sex + T_a + treatment + sex:T_a + sex:treatment	−91.4	7.8
	sex + T_a + treatment + sex:T_a + T_a:treatment	−90.1	9.1
	sex + T_a + treatment + sex:treatment + T_a:treatment	−86.8	12.4
BMR	**sex + treatment**	**−57.7**	**0.0**
	sex + T_a + treatment	−47.5	10.2
	treatment	−45.9	11.7
	sex + T_a + treatment + sex:T_a	−39.4	18.3

Body mass (g, BM); whole day activity (counts, WDA); daytime activity (counts, DA); night-time activity (counts, NA); resting metabolic rates ($\dot{V}O_2$, ml g^{-1} h^{-1}, RMR); basal metabolic rates ($\dot{V}O_2$, ml g^{-1} h^{-1}, BMR). The best-fit model for each variable is indicated in bold.

treatment at age ∼230 days (**Figure 2**; females: *p*-value = 0.001; males: *p*-value = 0.009). The reverse was found when antechinus were moved from the warm to the cold treatment (**Figure 2**; females: *p*-value < 0.0001; males: *p*-value = 0.003).

Similar results were found for daytime and nightly activity, such that there were no significant differences among sexes and treatment groups (**Figure 2**; *p*-values ranged from 0.262 to 1.000). Only males displayed a significant increase in daytime activity levels when transferred from the cold to warm treatment (**Figure 2B**; *p*-value < 0.0001) and a decrease from the warm to cold treatment (**Figure 2B**; *p*-value < 0.0001). While males in both treatment groups did not alter nightly activity levels after being transferred to the new treatment at age ∼230 days (**Figure 2B**; *p*-values ranged from 0.643 to 1.000), females increased nightly activity when moved from the cold to the warm treatment (**Figure 2A**; *p*-value = 0.001) and decreased nightly activity from the warm to the cold treatment (**Figure 2A**; *p*-value = 0.001).

Metabolism

Mass-specific resting metabolic rates (RMR) throughout the thermoregulatory curve were best explained by sex, T_a, treatment and interactions between these variables (**Table 1**). In general, RMR increased with decreasing T_a for both sexes and all treatment groups (**Figure 3**). For all treatment groups, females displayed higher RMR than males (**Figure 3**; *p*-values ranged from <0.0001 to 0.032). For both females and males, RMR was higher in the cold treatment group in comparison to the warm treatment group (**Figure 3**; all *p*-values < 0.0001) and RMR decreased when individuals were transferred from the cold treatment to the warm treatment (**Figure 3**; all *p*-values < 0.0001). Only for females, RMR significantly increased after individuals were transferred from the warm treatment to the cold treatment (**Figure 3A**; *p*-value < 0.0001). In contrast, there was no significant change in RMR after males were placed into the cold treatment after the warm treatment (**Figure 3B**; *p*-value = 0.635).

Only the variables sex and treatment were included in the best-fit model for BMR (**Table 1**). Similarly to RMR, females (warm = 1.05 ± 0.04 ml g^{-1} h^{-1}, body mass = 26.4 ± 2.7 g, *n* = 4; cold = 1.25 ± 0.03 ml g^{-1} h^{-1}, body mass = 27.6 ± 2.9 g, *n* = 4; warm-cold = 1.14 ± 0.05 ml g^{-1} h^{-1}, body mass = 28.4 ± 2.9 g, *n* = 4; cold-warm = 0.88 ± 0.06 ml g^{-1} h^{-1}, body mass = 33.1 ± 4.4 g, *n* = 4) had higher BMR for all treatments in comparison to males (warm = 0.91 ± 0.01 ml g^{-1} h^{-1}, body mass = 42.7 ± 2.8 g, *n* = 4; cold = 1.08 ± 0.07 ml g^{-1} h^{-1}, body mass = 47.9 ± 3.2 g, *n* = 4; warm-cold = 0.99 ± 0.02 ml g^{-1} h^{-1}, body mass = 40.0 ± 6.2 g, *n* = 4; cold-warm = 0.81 ± 0.03 ml g^{-1} h^{-1}, body mass = 51.7 ± 4.5 g, *n* = 4; **Figure 3**; all *p*-values = 0.0006). Further, for both sexes BMR was greater in the cold treatment group in comparison to the warm treatment group (**Figure 3**; *p*-values < 0.0001 for females and males) and decreased when individuals were transferred to the warm treatment (**Figure 3**; *p*-values < 0.0001 for females and males). However, BMR did not change for individuals of both sexes from the warm treatment group after acclimatization to the cold treatment (**Figure 3**; *p*-values ranged from 0.239 to 0.277).

DISCUSSION

Rearing yellow-footed antechinus under different thermal conditions resulted in changes in morphology and function and the strongest effects were seen in their behavior and physiology. Warmer temperatures resulted in greater daily activity levels and lower MRs. Such changes are typical for many mammals as seasons shift from winter to summer. However, our data suggest that at least physiologically, individuals raised in warm conditions may have less phenotypic flexibility in regard to changing temperature regimes. Our results also revealed that

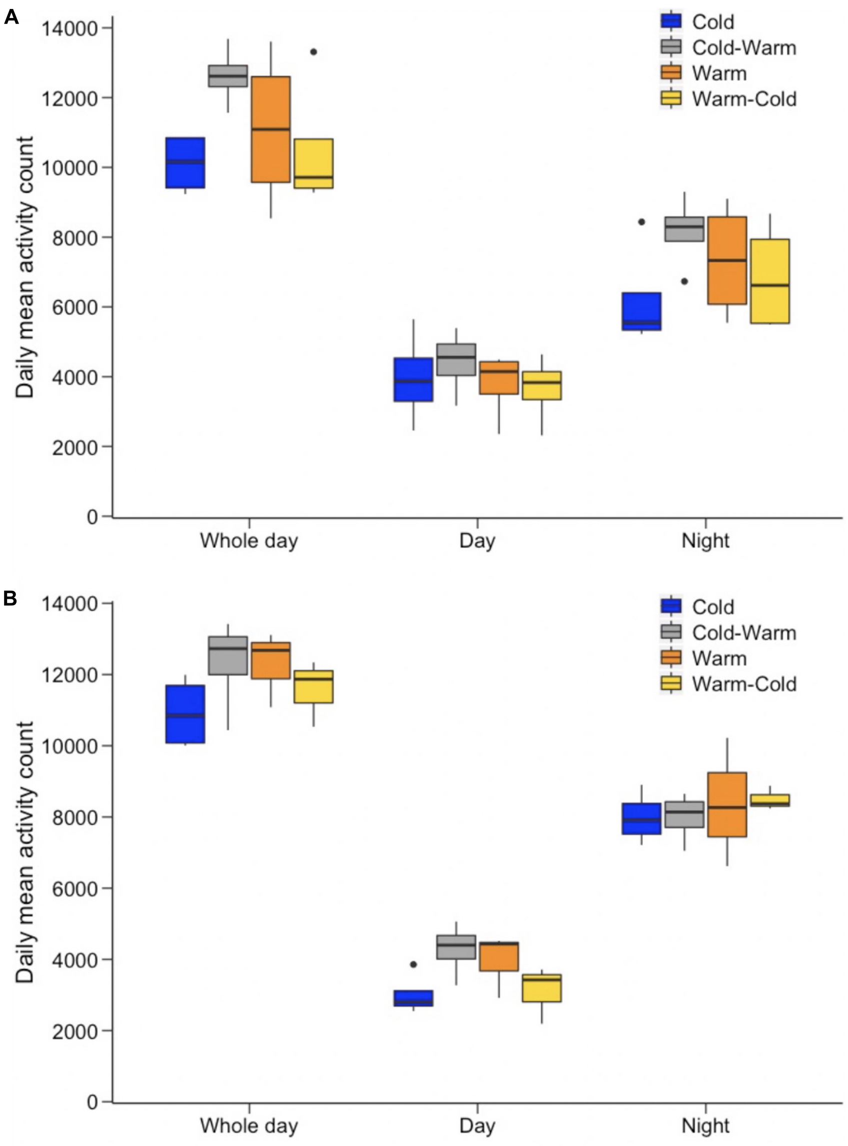

FIGURE 2 | Mean activity count for **(A)** females and **(B)** males for each of the treatment groups (represented by different colors as shown in the key). Whole day, daytime, and night-time activity are presented separately.

growth rates in antechinus are not strongly influenced by temperature, but perhaps more by sex and food availability.

Displaying sexual dimorphism, males are typically larger for most antechinus species (Naylor et al., 2008), which can also be seen in the juvenile growth rates in the current study. Similarly to a previous study on brown antechinus (*Antechinus stuartii*; Westman et al., 2002), the yellow-footed antechinus in our study showed a steeper linear increase in body mass initially, which flattened out as they aged producing a sigmoidal growth curve. However, males continued to increase body mass more significantly than females throughout their life. In relation to acclimation temperatures and reversal, body mass for either male or female antechinus was not affected. In contrast, in accordance with Bergmann's rule, some birds and mammals display higher body mass after cold acclimation in captivity and also during winter in the wild in comparison to summer (Meiri and Dayan, 2003; Zhang and Wang, 2007; Zhu et al., 2012; Wu et al., 2015; Hu et al., 2017). Such increases in body mass as a result of cold acclimation may be due to an increase in food intake (Hu et al., 2017). As the amount of food that the antechinus in our study consumed did not change throughout the experiment and was the same for both treatment groups, they may not have had the means to significantly alter their body mass. However, some other mammals also display no changes in body mass when acclimated to warm and cold environments. and instead increase their thermogenic capacity rather than body mass to deal with colder temperatures (Steffen and Roberts, 1977; Wang et al., 2006). The approaches of

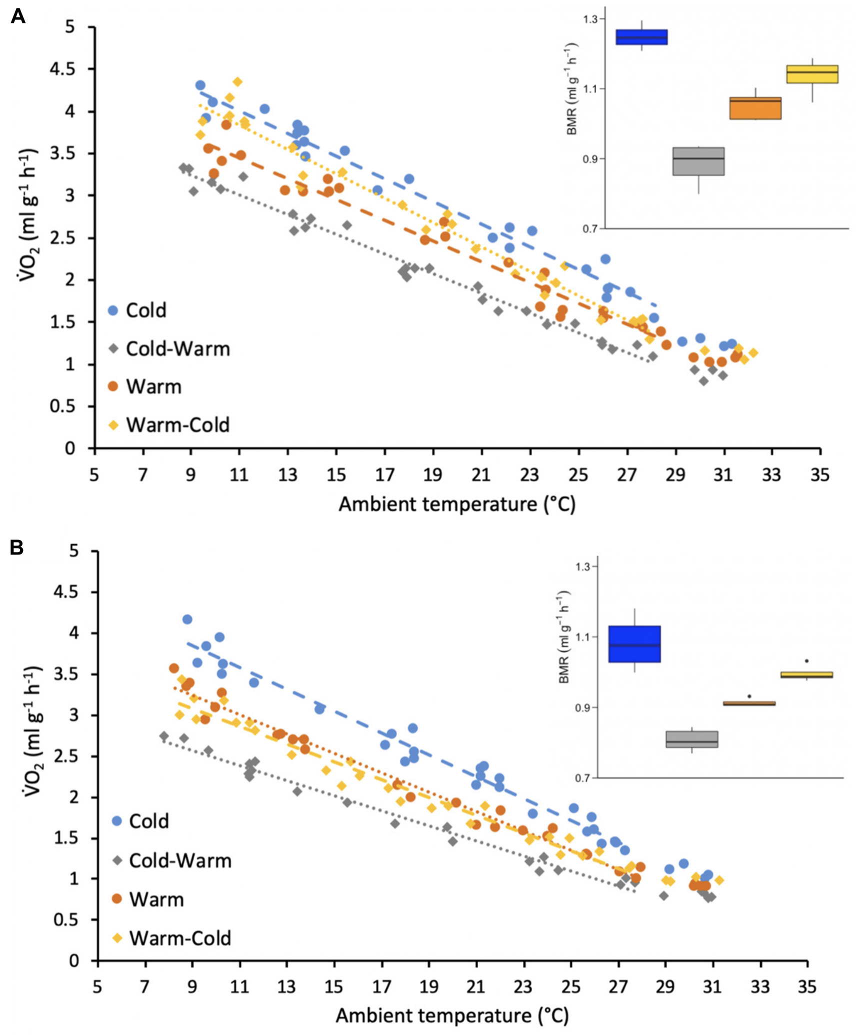

FIGURE 3 | Resting metabolic rates (RMR measured $\dot{V}O_2$, ml g^{-1} h^{-1}) of adult **(A)** female and **(B)** male individual antechinus at different ambient temperatures (°C). Each of the treatment groups are represented by a different color as shown by the key. The circles and dashed lines represent the initial treatment groups and the diamonds and the dotted lines represent the treatment groups after reversal. The points forming the regression lines represent resting metabolic rates (RMR) and the points without a line represent basal metabolic rates (BMR). The inset boxplots represent the BMR (ml g^{-1} h^{-1}) measured for each treatment group and sex.

increasing body mass and/or increasing thermogenic capacity to colder conditions are only feasible for animals with diets that permit continual feeding throughout the year. For insectivorous species such as antechinus, this is not possible as their main food source is greatly reduced in the cold. Extensive reviews on Bergmann's rule have found mixed support for temperature being the main factor influencing body size in mammals, and have suggested that other factors such as food availability are more important (Alhajeri and Steppan, 2016; Gohli and Voje, 2016). As antechinus are known to increase torpor use when exposed to cold conditions to save energy (Geiser, 1988; Parker et al., 2019), it is likely that they increased torpor use when exposed to the cold treatment. Therefore, this strategy, along with changes in activity patterns, may reduce body mass loss under cold conditions and explain why there was no difference between the treatment groups in our study.

Warmer temperatures typically lead to increased activity levels in endotherms (Vickery and Bider, 1981), which was the general observation for the antechinus in the current study. Further, antechinus did alter their activity patterns after being placed into a new temperature regime, becoming more active in the warm and less active in the cold treatment. There is often a threshold T_a above which the energetic cost of activity becomes prohibitively expensive. Therefore, many endotherms will reduce activity and remain resting at not only extreme cold temperatures, but also at extreme hot temperatures (Randall and Thiessen, 1980). One way animals can avoid extremely high T_a is by being more active at night when temperatures are cooler. Greater nocturnal activity in both sexes is already typical for antechinus and they are often regarded as one of many nocturnal marsupials, although recent studies have revealed extensive daytime activity as well (Stawski et al., 2016). This phenotypic flexibility found for activity patterns for antechinus may be beneficial under future climate scenarios. Such changes in activity patterns in response to variation in T_a also often coincide with shifts in an individual's daily energy management strategies, particularly metabolism.

The differences found in both RMR and BMR between the cold and warm treatment groups reveal that even small temperature differences during development can have long-lasting effects on an individual's physiology. As mass-specific RMR has been shown to decrease as young mature until they reach adult size (Holloway and Geiser, 2000), it is important to note that antechinus have already developed physiological thermoregulation before they have been weaned (Westman et al., 2002). Therefore, as we measured metabolism in adult antechinus, there should be no contribution from development on the measured MR. Importantly, variation in the level of RMR can impact both fitness and survival during development and throughout the life of an organism. For example, a low RMR decreases the amount of energy an animal needs and will be optimal for survival when foraging returns are limited and/or foraging is risky (Larivée et al., 2010). However, a high RMR can also be advantageous in order to have more energy for activities such as mating and to increase cold tolerance and thermogenic capacity (Liknes and Swanson, 1996; Stawski et al., 2017a). Therefore, it would be beneficial if animals are able to adjust RMR in response to climate change and also more generally throughout the year in order to cope with prevailing weather and food conditions.

For many endotherms and the antechinus in our study, a reduction in overall T_a as a result of winter conditions or acclimation experiments often results in an increase in BMR (Haim et al., 1991; McDevitt and Speakman, 1994; Liknes and Swanson, 1996; Corp et al., 1997; Anderson and Jetz, 2005; McKechnie et al., 2007; Zhao et al., 2010, 2014; Bozinovic et al., 2011; Chi and Wang, 2011; Boratyński et al., 2016). This solution provides a way for endotherms to use less energy for thermoregulation in the colder winter months and to enhance their cold tolerance, as they need to use more energy once the temperature drops below the TNZ and food availability is typically reduced in cold conditions. Such changes in thermal physiology are particularly important for those species that do not alter their insulation and/or body mass in response to changing seasons (Haim et al., 1991), such as the antechinus in the current study. Further, the antechinus perhaps compensated for having higher RMR and BMR under cold conditions by having lower activity levels. On the other hand, moderately warmer temperatures require either less heat production or the employment of heat loss mechanisms such as evaporation (Anderson and Jetz, 2005; Zhao et al., 2010). Therefore, a low BMR in this case may be more beneficial, as displayed by the warm-acclimated antechinus in the current study. Although green ringtail possums can store heat at high temperatures, likely to avoid evaporative cooling (Krockenberger et al., 2012), T_b cannot continually increase as this would result in death, and heat storage is not an effective option for small species like antechinus. Therefore, while this strategy would be suitable for heat waves lasting several hours, it would not suffice during the longer heat waves that are already occurring. The physiological flexibility of shifting to a lower BMR and RMR may instead provide the means to cope with long-term increases in T_a.

CONCLUSION

As a shift in photoperiod historically coincided with a regular change in season and environmental conditions, it provided a reliable signal for a seasonal phenotypical change. However, this is unlikely to be the case with the increasing unpredictability of weather conditions. Therefore, while some species will be able to adapt to a changing climate by altering the timing of their reproductive and subsequent development periods (Ozgul et al., 2010; Hoffmann and Sgrò, 2011), species such as the antechinus in our study seem to be unable to do so as the timing of their reproductive period is based on photoperiod, a fixed environmental variable, rather than changes in the weather. Consequently, it is important that each generation is able to adapt to a changing environment via alterations of phenotypic traits. For a short-lived species such as antechinus, there could also be selection to flexibly adjust these traits during their developmental period, which they appear capable of for at least activity and energetics. Interestingly, it appears that the cold-acclimated rather than warm-acclimated individuals have more phenotypic flexibility in regard to RMR and BMR. As adult antechinus, especially males, spend most of their life in winter conditions it is perhaps beneficial for them to adapt more readily to changes during winter rather than summer. The capability of altering the metabolic curve for thermoregulation in response to a change in climate will be particularly advantageous.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Committee of University of New England.

AUTHOR CONTRIBUTIONS

CS and FG conceived the project and wrote the manuscript. CS collected and analyzed the data.

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frontiers in Physiology

ORIGINAL RESEARCH
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Mitochondrial Costs of Being Hot: Effects of Acute Thermal Change on Liver Bioenergetics in Toads (*Bufo bufo*)

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Global climatic warming is predicted to drive extreme thermal events, especially in temperate terrestrial environments. Hence, describing how physiological parameters are affected by acute temperature changes would allow us to understand the energy management of organisms facing such non-predictable and constraining events. As mitochondria play a key role in the conversion of energy from food into ATP but also produce harmful reactive oxygen species, the understanding of its functioning is crucial to determine the proximal causes of potential decline in an animal's performance. Here we studied the effects of acute temperature changes (between 20 and 30°C) on mitochondrial respiration, ATP synthesis rate, oxidative phosphorylation efficiency (ATP/O), and H_2O_2 generation in isolated liver mitochondria of a terrestrial ectotherm, the common toad (*Bufo bufo*). Using succinate as the respiratory substrate, we found that the mitochondrial rates of oxygen consumption, ATP synthesis, and H_2O_2 generation increased as the temperature increased, being 65, 52, and 66% higher at 30°C than at 20°C, respectively. We also found that the mitochondrial coupling efficiency (ATP/O) decreased, while the oxidative cost of ATP production (H_2O_2/ATP ratio) increased. The present results further indicate that between 40 and 60% of temperature effects on mitochondrial ATP production and H_2O_2 generation was *at minima* driven by an action on the oxidative capacity of the mitochondria. These results suggest that *B. bufo* may need to allocate extra energy to maintain ATP production and protect cells from oxidative stress, reducing the energy allocable performances.

Keywords: H_2O_2 release, oxygen consumption, ATP synthesis, amphibian, temperature, mitochondrial efficiency

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INTRODUCTION

Long-term records in climatic data indicate that the global surface temperature has increased by 0.2°C per decade in the past 30 years (Intergovernmental Panel On Climate Change [IPCC], 2014). However, it is important to differentiate between "climate" which is observed year after year and "weather" that is much less predictable. Predictions of ever more extreme weather patterns are associated with climate change, especially in terrestrial environments, such as higher frequency and duration of heat waves and heavy rainfall events during the summer in North America and Europe (Fischer and Knutti, 2013; Ummenhofer and Meehl, 2017). Both theoretical predictions and experimental data show that extreme weather events impact biodiversity and shift the geographic species distribution (Bellard et al., 2012). In addition to the anthropic use of land and water, which can induce rapid and stochastic environmental changes, weather (not climate) will probably be the most challenging characteristic of climate change facing terrestrial animals. Freshwater ecosystems will also be strongly impacted by extreme events with floods or drought-induced variation of temperature, pollution, and oxygen content (Xia et al., 2015). The aquatic populations must behaviorally and/or physiologically respond to optimize the performance of individuals under these new conditions (Woodward et al., 2016).

Temperature is one of the most prominent abiotic parameters. Environmental temperature governs the body temperature of ectothermic animals which in turn determines their biochemical reactions, cellular metabolism, and physiological functions. Thus, the thermal dependence of biological activities and performance puts ectothermic organisms at risk due to the rise in extreme thermal events driven by global climatic warming, even if major drivers of extinction are hard to predict (Kearney et al., 2009). In most animals, including ectothermic species, aerobic metabolism is fundamentally important in supplying the energy that is needed for cellular activity, performance, and animal life. By providing most of the ATP needed for cellular activities, the mitochondria constitute the main physiological link between environmental resources and animal performance. Reactive oxygen species (ROS) are also inevitable by-products of mitochondrial aerobic metabolism, a biochemical process that occurs when electrons leak from the electron transport chain during oxidative phosphorylation to directly react with molecular oxygen (Brand, 2016). Thus, a key reason for focusing on mitochondrial functioning is its importance in supplying energy and regulating the oxidative balance that are involved in many life history traits and trade-offs (Monaghan et al., 2009; Seebacher et al., 2010; Salin et al., 2015; Schulte, 2015). Mitochondrial oxidative phosphorylation and ROS generation are known to be directly influenced by temperature (Cassuto, 1971; Abele et al., 2002; Heise et al., 2003; Chamberlin, 2004; Paital and Chainy, 2014; Chung and Schulte, 2015; Wiens et al., 2017), generating similar shapes of performance curves with those of many biochemical and physiological processes (Pörtner et al., 2007; Schulte, 2015). Performance increases as temperatures rise, followed by a plateau when the processes reach thermal optimum, followed by a steep decline at higher temperatures (Pörtner et al., 2007; Schulte, 2015). Therefore, understanding the mitochondrial responses in these organisms during extreme events may be crucial to determine the proximal causes of potential population decline (Heller and Zavaleta, 2009).

An increase in mitochondrial activity during the first ascendant phase of the thermal performance curves would be associated with an increased output of cellular energy in the form of ATP and thus would be beneficial for animal performance. However, such beneficial effect of warming on mitochondrial activity will be counterbalanced by an increase in energy cost associated with the maintenance of membrane potential due to increased membrane fluidity (Dufour et al., 1996; Chamberlin, 2004; Brown et al., 2012), which would decrease the mitochondrial coupling efficiency (Monternier et al., 2014). The thermal effects on mitochondrial ROS generation can also be compared: (i) it can be increased following an increase in mitochondrial oxidative activity, as discussed in the free radical theory of aging (Barja, 2013) or (ii) it can be decreased following increased membrane fluidity as suggested by the "uncoupling to survive" hypothesis (Brand, 2000). In the present paper, we deciphered the mitochondrial mechanisms involved in an acute (without acclimation) increase of temperature in an ectotherm: the common toad (*Bufo bufo*). The aim of this work was to understand how oxygen consumed by the mitochondria was allocated between the maintenance (proton leakage, H_2O_2 generation) and productive (ATP synthesis) costs of mitochondrial energy metabolism.

MATERIALS AND METHODS

Animals

We chose the common toad as a model since it is one of the most common amphibians in Europe having a wide latitudinal and altitudinal distribution (Borkin and Veith, 1997) as well as a large variation in body temperature. The body temperatures range from 12 to 19°C at night and from 28 to 34°C when in daytime retreats or abroad (Meek and Jolley, 2006). Fifteen male toads (*B. bufo*) were collected in the spring (March) from a pond located 40 km south of Lyon, France (4°92 E, 45°50' N). The breeding number was estimated to be over 3,500 males. The animals were individually housed in a box maintained at 20°C for 1 week. The mean body mass (±SD) was 39.9 ± 3.1 g at the time of the experiments.

Mitochondrial Isolation

Mitochondria were isolated in an ice-cold isolation buffer (250 mM sucrose, 1 mM EGTA, and 20 mM Tris-HCl, pH 7.4) from three livers per preparation, with all steps performed at 4°C (Salin et al., 2012; Roussel et al., 2015). A total of five independent mitochondrial preparations were done in the present study. Briefly, 2–3 g of liver (mean tissue mass was 0.93 ± 0.06 g per animal) was homogenized with a Potter–Elvehjem homogenizer (three passages). The homogenate was centrifuged at 800 × *g* for 10 min. The resulting supernatant was centrifuged at 1,000 × *g* for 10 min, filtered through cheesecloth, and re-centrifuged at 8,700 × *g* for 10 min to pellet mitochondria. The liver mitochondrial pellet was washed twice by suspension in the isolation buffer and centrifuged at 8,700 × *g* for 10 min. The protein concentration of the mitochondrial suspension was determined by the biuret method with bovine serum albumin as a standard. The toad mitochondrial preparations contained a dark pigment which absorbed at 540 nm, and the absorbance of the same volume of mitochondria in water containing 0.6% deoxycholate and 3% NaOH was subtracted.

Mitochondrial ATP Synthesis and Oxidative Phosphorylation Efficiency

Liver mitochondria (1.5 mg/ml) were incubated in 500 μM respiratory medium (120 mM KCl, 1 mM EGTA, 5 mM KH_2PO_4, 2 mM $MgCl_2$, 0.3% of essentially free fatty acid bovine serum albumin, and 3 mM HEPES adjusted to pH 7.4) supplemented with glucose (20 mM) and hexokinase (1.5 U/ml). The air-saturated medium was assumed to contain 521, 479, and 437 nmol of O/ml at 20, 25, and 30°C, respectively. Oxygen consumption and ATP synthesis rates were performed at 20, 25, or 30°C using succinate (5 mM) as respiratory substrate in the presence of rotenone (5 μM). The mitochondrial ATP synthesis was initiated by the addition of 100, 25, or 10 μM and followed by glucose-6-phosphate accumulation using an ATP-regenerating system (hexokinase plus glucose) as previously described for frogs (Salin et al., 2012; Roussel et al., 2015). Briefly, after recording the phosphorylating respiration rate, four 100-μl samples of mitochondrial suspension were withdrawn from the suspension every 2 min and immediately quenched in perchloric acid solution (10% $HClO_4$ and 25 mM EDTA). After centrifugation of the denatured protein and neutralization of the resulting supernatant, the glucose-6-phosphate content of the samples was measured by spectrophotometry according to Lang and Michal (1974).

The maximal oxidative activity of the electron transport system (ETS) was measured with a Clark oxygen electrode (Rank Brother Ltd., United Kingdom) in a stirred and closed chamber with a volume of 500 μl, thermostatically controlled at three different temperatures (20, 25, or 30°C). The maximal fully uncoupled respiration rate, associated with the maximal ETS activity, was measured on mitochondria respiring on succinate (5 mM) in a respiratory buffer supplemented with 5 μM rotenone, 2 μg/ml oligomycin, and 2 μM carbonyl cyanide *p*-tri-fluoro-methoxy-phenyl-hydrazone (FCCP).

Mitochondrial Radical Oxygen Species Production

The measurement of mitochondrial H_2O_2 generation was performed with a Kontron fluorometer (model SFM-25) in a stirred chamber of 1 ml volume, thermostatically controlled at three different temperatures (20, 25, or 30°C). Liver mitochondria (0.4 mg/ml) were incubated in a respiratory medium supplemented with 5 U/ml horseradish peroxidase and 1 μM Amplex red. The rate of mitochondrial H_2O_2 release was assessed following the linear increase in fluorescence ($\lambda_{\text{excitation}}$ = 560 nm and $\lambda_{emission}$ = 584 nm) in the presence of succinate (5 mM) and then after the addition of ADP (100 μM). The fluorescent signal was calibrated using a standard curve prepared with known concentrations of H_2O_2.

Thermal Sensitivity of Mitochondrial Metabolism (Q_{10})

The temperature coefficient Q_{10} was calculated for mitochondrial fluxes (oxygen consumption, ATP synthesis, and H_2O_2 generation) using the following formula:

$$Q_{10} = (R_2/R_1)^{10/(T2-T1)}$$

where R_1 and R_2 denote the mitochondrial flux at higher (T_2) or lower (T_1) temperatures, respectively. The Q_{10} value was calculated for the temperature range of 20–25 and 25–30°C.

Statistical Analyses

The results are presented as mean ± SEM. A one-way repeated-measure analysis of variances (RM ANOVA) was performed to test the temperature effect on fluxes (oxygen consumption, ATP production, and H_2O_2 release) as well as free electron leak. Multiple linear regression was performed to test the relationships between the different parameters, with temperature as an independent variable. When variances in homogenization and/or homoscedasticity were not observed, non-parametric Friedman test was used. The statistical analyses were performed using JMP 12 (SAS Institute Inc., Cary, NC, United States). A 5% ($p = 0.05$) level of significance was used in all of the tests.

RESULTS

ATP Synthesis and Oxidative Phosphorylation Efficiency

The basal non-phosphorylating rates of oxygen consumption increased with increasing temperature (basal state; **Table 1**). The maximal rates of ATP synthesis and corresponding oxygen consumption increased from 20 to 25 and to 30°C (active state; **Table 1**). The respiratory control ratio (RCR) was not significantly affected by temperatures ($RCR_{20°C} = 3.14 \pm 0.18$; $RCR_{25°C} = 3.15 \pm 0.14$; $RCR_{30°C} = 3.05 \pm 0.21$). **Figure 1** shows the effect of temperature on the linear relationship between the rates of ATP synthesis and of oxygen consumption in mitochondria working at different steady-state rates. There was no significant effect of temperature on the slope values ($ATP/O_{20°C} = 1.53 \pm 0.13$; $ATP/O_{25°C} = 1.47 \pm 0.07$; $ATP/O_{30°C} = 1.47 \pm 0.11$), indicating that these were parallel relations. Since the basal non-phosphorylating respiration rates (the intercepts with the *x*-axis) were significantly affected by temperature (basal state; **Table 1**), the linear relations were significantly shifted to the right as the temperature increased (**Figure 1**). Consequently, more oxygen was consumed at any steady-state rates of ATP production when the temperature increased, indicating that mitochondrial coupling efficiency decreased with increasing temperature. This is clearly illustrated in **Figure 1B** where the coupling efficiency was calculated at the maximal rate of ATP synthesis measured at 20°C, the highest common mitochondrial ATP production between thermal conditions. Interestingly, there was a close correlation between FCCP-induced maximal activity of ETS and the maximal rate of ATP synthesis and efficiency, indicating that at least 40% of the temperature effects on mitochondrial ATP production and efficiency were driven by an action on the oxidative capacity of the mitochondria (**Figure 2**).

Mitochondrial H_2O_2 Generation and Oxidative Cost of ATP Synthesis

There was a significant positive relationship between oxygen consumption and H_2O_2 generation in the mitochondria respiring on succinate under both basal non-phosphorylating and active phosphorylating states (**Figure 3**). The higher the respiration rates, the higher the H_2O_2 production. Hence, the mean values

TABLE 1 | Effect of temperature on mitochondrial metabolism and H_2O_2 production.

	Temperature (°C)			Statistical analysis	
	20	25	30	*F*-values	*P*-values
Oxygen consumption rate (nmol O min^{-1} mg protein $^{-1}$)					
Basal state	3.9 ± 0.4	5.2 ± 0.7*	7.0 ± 1.0*†	15.7	
Active state	12.8 ± 1.7	16.5 ± 2.5*	21.1 ± 2.7*†	38.2	
Maximal ETS	18.2 ± 2.5	20.9 ± 2.6	25.6 ± 3.6*†	9.6	
ATP synthesis rate (nmol ATP min^{-1} mg $protein^{-1}$)					
Active state	13.6 ± 1.9	16.4 ± 2.3*	20.7 ± 3.3*†	15.2	$p < 0.01$
H_2O_2 generation rate (pmol H_2O_2 min^{-1} mg $protein^{-1}$)					
Basal state	133 ± 16	178 ± 17*	240 ± 24*†	38.8	$p < 0.0001$
Active state	73 ± 8	93 ± 9*	155 ± 8*†	255.5	$p < 0.0001$
Free electron leak (%H_2O_2/O)					
Basal state	3.3 ± 0.2	3.5 ± 0.2	3.5 ± 0.3	n.s.	n.s.
Active state	0.59 ± 0.04	0.60 ± 0.06	0.77 ± 0.08*†	8.5	$p < 0.05$

*The mitochondrial oxygen consumption rates and the H_2O_2 production were measured in isolated liver mitochondria fueled with succinate alone (basal state) or with ADP (active state) or with FCCP in the presence of oligomycin (maximal ETS, maximal activity of the electron transport system). Free electron leak is the percent of oxygen turned into H_2O_2 instead of H_2O (%H_2O_2/O). The values are expressed as means ± SEM (N = 5). *$p < 0.05$ versus 20°C; †$p < 0.05$ versus 25°C.*

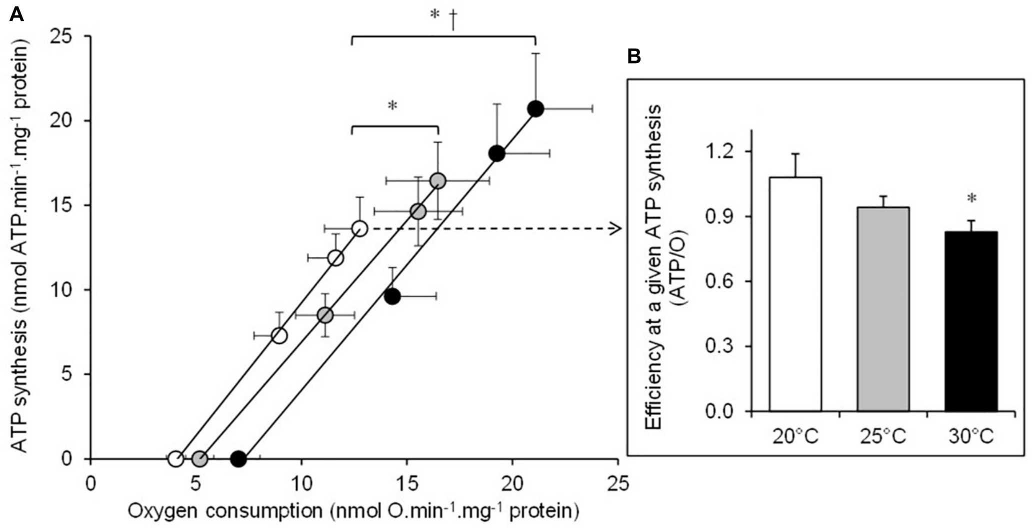

FIGURE 1 | The effect of temperature on the mitochondrial oxidative phosphorylating efficiency. **(A)** The rates of ATP synthesis and oxygen consumption were determined at 20°C (white circles), at 25°C (gray circles), and at 30°C (black circles) in the mitochondria isolated from the liver of toad and respiring on succinate. Mitochondrial fluxes were titrated with increasing concentrations of ADP in the presence of glucose and hexokinase (see section "Materials and Methods" for more details). Values are means ± SEM for *N* = 5 independent mitochondrial preparations. **(B)** The coupling efficiency calculated at the highest common ATP synthesis rate between the three temperatures (13.6 nmol ATP min^{-1} mg^{-1} protein). Shortly, oxygen consumption rates were calculated at this highest common ATP synthesis rate by using individual linear relation curves. Then, the resulting effective ATP/O ratios were calculated at 20°C (white bars), at 25°C (gray bars), and at 30°C (black bars). The values are means ± SEM for *N* = 5 independent mitochondrial preparations. * and † mean significantly different from 20 and 25°C, respectively ($p < 0.05$).

of H_2O_2 generation significantly increased between 20 and 25 and to 30°C under both basal and active states (**Table 1**). The electron leak (%H_2O_2/O) was not affected by temperatures in the basal non-phosphorylating state. The electron leak was not significantly changed between 20 and 25°C but was significantly higher at 30°C than at 20 and 25°C in the active phosphorylating state. There was also a significant positive relationship between H_2O_2 generation and ATP synthesis rates (**Figure 4A**) with temperature effect (values at 30°C are significantly higher than those at 20 and 25°C). This temperature effect explains the higher oxidative cost of ATP production (H_2O_2/ATP ratio) at 30°C compared with those at 20 and 25°C (**Figure 4B**).

Thermal Sensitivity of Mitochondrial Metabolism and H_2O_2 Production

Thermal sensitivity (Q_{10}) was not significantly different for any aspect of mitochondrial function, except for H_2O_2 production under the active phosphorylating state (**Figure 5**). Mitochondrial H_2O_2 production under the active state exhibited a 1.7-fold

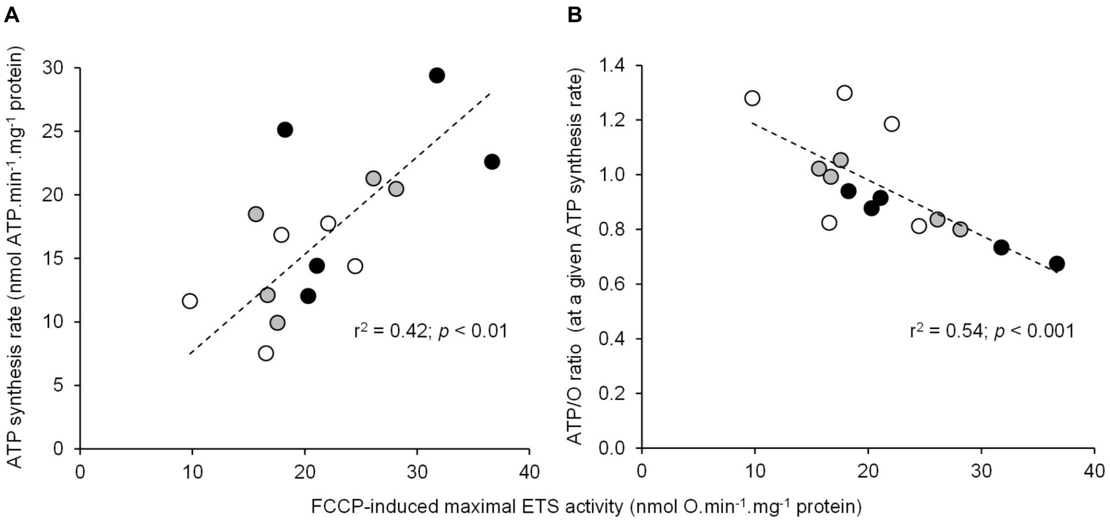

FIGURE 2 | Relationship between the individual maximal oxidative activities and the ATP synthesis rate or coupling efficiency. The correlation of mitochondrial FCCP-induced maximal ETS activity using succinate as the respiratory substrate with **(A)** the maximal rate of ATP synthesis (mean values are reported in **Table 1**) or **(B)** the coupling efficiency (ATP/O ratio) calculated at the highest common ATP synthesis rate (mean values are shown in **Figure 1B**). Individual values show five independent mitochondrial preparations from the liver of toad measured at 20°C (white circles), at 25°C (gray circles), and at 30°C (black circles).

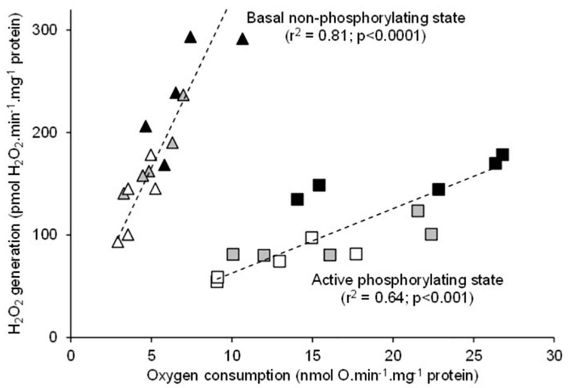

FIGURE 3 | Relationship between the individual H_2O_2 production and the mitochondrial oxygen consumption rates during fueling with succinate alone (basal non-phosphorylating state) or with succinate and ADP (active phosphorylating state). The individual values show five independent mitochondrial preparations from the liver of toad measured at 20°C (white circles), at 25°C (gray circles), and at 30°C (black circles).

increase in Q_{10} value compared with the values of other mitochondrial parameters (**Figure 5**).

DISCUSSION

The data presented here show that the rapid increase in temperature induces higher mitochondrial rates of oxygen consumption, ATP synthesis, and H_2O_2 production. These results are in accordance with the acute temperature effects on oxidative phosphorylation activity and ROS production reported in several ectothermic species (Cassuto, 1971; Abele et al., 2002; Heise et al., 2003; Paital and Chainy, 2014; Chung and Schulte, 2015; Wiens et al., 2017). However, high temperatures also decrease the mitochondrial coupling efficiency (ATP/O). This result indicates that the beneficial effect of increasing temperature on mitochondrial ATP synthesis is associated with an energy cost mediated by a loss of efficiency. These results indicate that the mitochondrial energy transduction system has to consume more oxygen (+ 20 ± 11% at 25°C and + 37 ± 13% at 30°C) so as to oxidize more substrates to sustain the production of a given amount of ATP. The negative impact of temperature on mitochondrial coupling efficiency is likely explained by a thermal effect on the fluidity of the inner membrane and a subsequent increase in proton permeability at high temperature (Chamberlin, 2004; Brown et al., 2007, 2012; Monternier et al., 2014; Power et al., 2014; Chung and Schulte, 2015; Jarmuszkiewicz et al., 2015; Zoladz et al., 2016). Nevertheless, our data also indicate that the loss of efficiency is also partly ascribed to a thermal stimulation of the activity of the ETS (**Figure 2B**). This result is in agreement with the previously reported negative link between the mitochondrial oxidative capacities and the efficiency of oxidative phosphorylation (Nogueira et al., 2001; Romestaing et al., 2008; Roussel et al., 2018). Mechanistically, an increased activity of the ETS would build up a higher membrane potential, triggering an increase in the voltage-dependent proton leakage and its negative control over the effective coupling efficiency (Brand et al., 1993; Roussel et al., 2004; Brand and Nicholls, 2011). Notwithstanding the underlying mechanisms, the present data highlight an extra energy need to generate cellular energy during hot weather events, suggesting that the potential energy allocated to performance such as growth or reproduction will be more limited. These elements provide a cellular mechanism of the "metabolic meltdown" phenomenon in ectotherms recently developed by Huey and Kingsolver (2019).

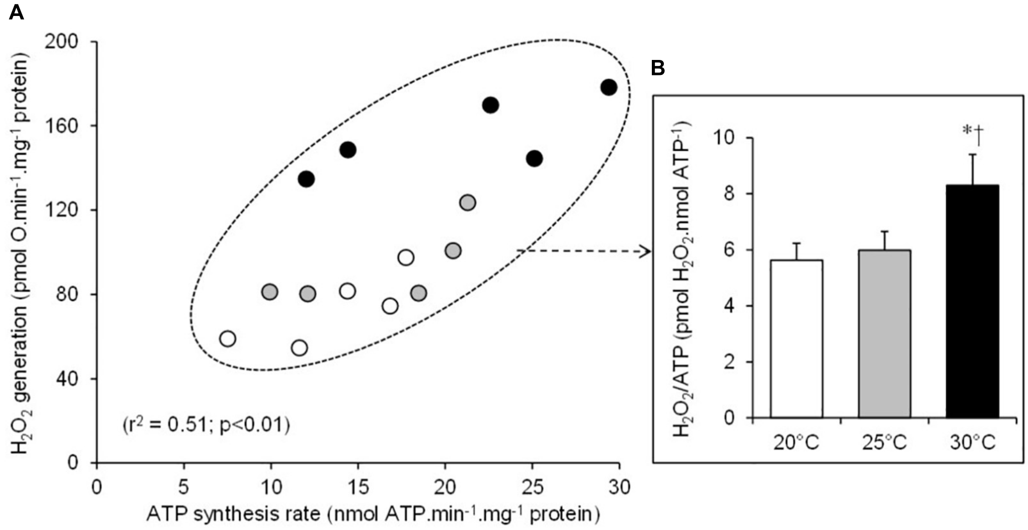

FIGURE 4 | Relationship between the individual H_2O_2 production and the mitochondrial ATP synthesis rates **(A)**. The individual values show five independent mitochondrial preparations from the liver of toad measured at 20°C (white circles), at 25°C (gray circles), and at 30°C (black circles). **(B)** The oxidative cost of ATP production (% H_2O_2/ATP ratio) calculated at 20°C (white bars), at 25°C (gray bars), and at 30°C (black bars). The values are means ± SEM for $N = 5$ independent mitochondrial preparations. * and † mean significantly different from 20 and 25°C, respectively ($p < 0.05$).

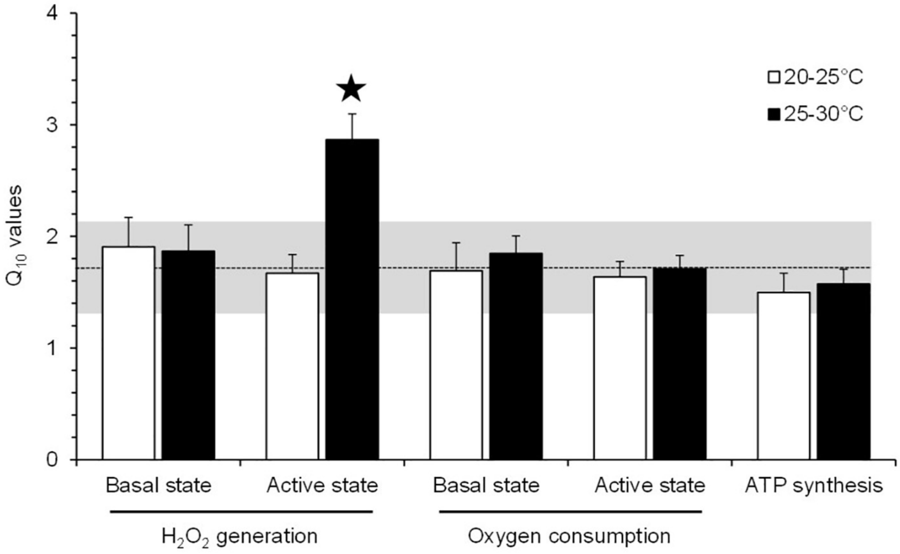

FIGURE 5 | Q_{10} values for the effect of acute temperature change on the mitochondrial parameters of toad liver. The values are means ± SEM for $N = 5$ independent mitochondrial preparations. *Significantly different from all of the other Q_{10} values ($p < 0.05$). The dashed line represents the mean of Q_{10} values for all mitochondrial parameters (±SD is represented by the horizontal gray-shaded rectangle), excluding the $Q_{10(25-30°C)}$ value for the H_2O_2 production under an active phosphorylating state.

The H_2O_2 generation in toad liver mitochondria is also positively correlated to the rates of oxygen consumption, with these two mitochondrial fluxes increasing as temperature increases. The increase of H_2O_2 production along with respiratory activity in response to an acute rise in temperature has been widely reported in mitochondria from ectothermic species (Abele et al., 2002; Heise et al., 2003; Paital and Chainy, 2014; Chung and Schulte, 2015; Wiens et al., 2017). In the present study, we used succinate, a $FADH_2$-linked substrate, which drives ROS production which is critically sensitive to proton-motive force (Korshunov et al., 1997; Miwa et al., 2003; Keller et al., 2004). As previously stated, the inner membrane proton leakage increases with increasing temperature, which should have led to a decreased inner membrane potential and thus ROS production. The present data have not verified this mechanism. This could be explained by the fact that the proton

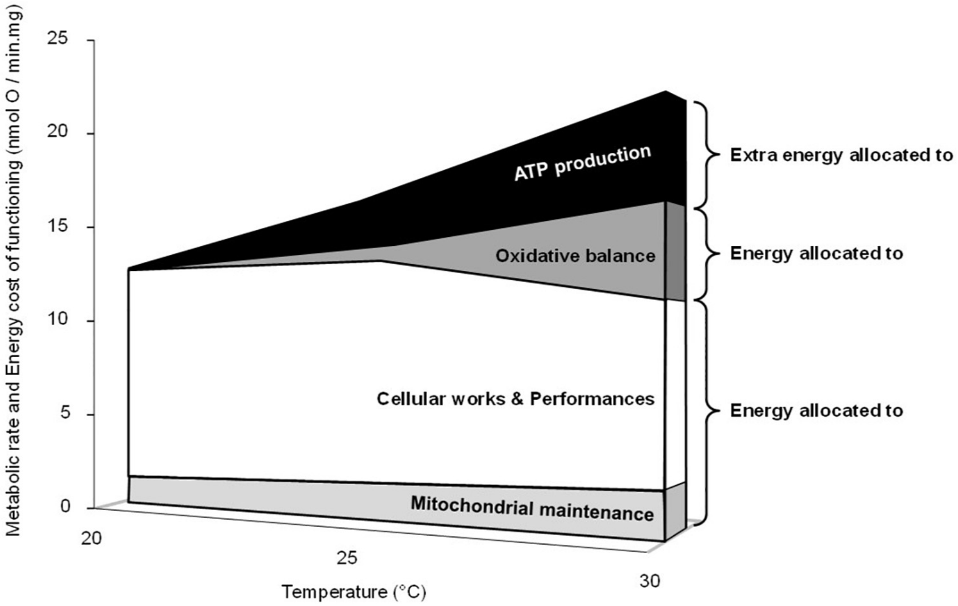

FIGURE 6 | Synoptic summary of the effects of acute temperature change on mitochondrial metabolism and energy costs. The upper limit in the figure represents the positive thermal effect on the maximal phosphorylating oxygen consumption rates (active states in **Table 1**), which is linearly related to the rates of ATP synthesis (**Figure 1A**). The "extra energy allocated to ATP synthesis" is related to the thermal decrease of the coupling efficiency and represents the extra oxygen needed to produce a given amount of ATP. It was calculated by using the percent changes in effective coupling efficiency measured at 25 and 30°C compared with values at 20°C (-15 and -22%, respectively; **Figure 1B**). The "energy allocated to oxidative balance," the "oxidative cost," is related to the thermal increase in H_2O_2 generation. It was calculated by using the mean value of the differences between the ratios%H_2O_2/O and%H_2O_2/ATP measured in the phosphorylating active state at 25°C or at 30°C and values measured at 20°C (+7 and +33%, respectively; **Table 1** and **Figure 4B**). "Mitochondrial maintenance" represents the need for oxygen and substrate consumption to counteract proton leakage and maintain the mitochondrial membrane potential value in active state. Even though the mitochondrial proton leakage is low in the phosphorylating active state, it still occurs, representing between 10 and 15% of the maximal phosphorylating oxygen consumption rate in liver mitochondria (Brand et al., 1993; Roussel et al., 2004, 2018). Here we used the fix value of 11%, arguing that the thermal increase in membrane conductance and subsequent proton leakage was counterbalanced by the thermal increase in the activity of the electron transport system (**Table 1**).

leak is not the only process to change with temperature; the activity of the electron transport chain also changes. These observations suggest that the thermal effect on the activity of the substrate oxidation system, which builds up membrane potential, compensates for the one on the proton leak, which consumes membrane potential. This is indirectly supported by the absence of thermal effect on the RCR. The RCR referred to the ratio between the active phosphorylating respiration rate, which is controlled by the activity of ATP turnover and substrate oxidation, and the basal non-phosphorylating respiration rate, which is mainly controlled by the activity of proton leak (Brand and Nicholls, 2011). Hence, no changes in RCR indicate that the activities of both the oxidative phosphorylation and the proton leak were similarly affected by temperature. This hypothesis is even more directly supported by data reporting no major decrease of membrane potential values with increasing temperatures in the mitochondria from several ectothermic species (Chamberlin, 2004; Trzcionka et al., 2008; Chung and Schulte, 2015). Thus, the thermal increase in H_2O_2 production reported here was mainly driven by an increased electron flow through the ETS since the fractional electron leak (%H_2O_2/O ratio) was not altered by temperatures, at least in basal state and in active state at 20 and 25°C. However, an oxidative cost of ATP production (H_2O_2/ATP) appears at the highest temperature tested (30°C) when the mitochondria were functioning at their maximal phosphorylating rate. This thermal sensitivity of H_2O_2 production was explained, at least in part, by a significant increase in free electron leakage (%H_2O_2/O ratio) during the maximal phosphorylating activity. On the whole and notwithstanding the underlying mechanisms, such thermal effects would clearly represent an oxidative cost of the mitochondrial functioning in ectotherms facing an extremely hot weather event. Of note, the stability of RCR highlights that oxidative stress occurred well before high temperatures cause a failure in mitochondrial activity.

Figure 6 presents a synoptic summary of the subcellular benefits and the costs of acute temperature increase on liver oxidative metabolism in the common toad. At the mitochondrial level, the rate of ATP synthesis (the power of life) increased in the range of the temperature tested, which represents a clear beneficial effect of temperature. However, this beneficial effect was counteracted by at least two energy costs. The first cost is the decrease of mitochondrial efficiency, which implies extra oxygen consumption (the fuel for life) and so a higher quantity of substrate to produce a given amount of cellular energy in the form of ATP. At the level of an organism, this would drive an increased foraging activity to fulfill resource needs to maintain ATP homeostasis and animal performance. Hence, part of the initial benefits would have to be invested into locomotor activity. The second cost is the elevated H_2O_2 production and especially the increase in the oxidative cost of ATP production (H_2O_2/ATP ratio) at 30°C. This cost would trigger increased oxidative damage to cellular macromolecules. Hence, part of the initial energy benefits would have to be invested into oxidative defenses and/or repair systems to counteract these increased oxidative damages. After summing up the two main costs, the loss of efficiency and the rise in H_2O_2 production, the energy costs associated with warming become higher than the initial benefit of having increased metabolic power at elevated temperatures. Indeed the energy left over to perform cellular activity becomes lower at 30°C than at 20 or 25°C (**Figure 6**). Interestingly, the increase of H_2O_2 production along with the decrease in coupling efficiency in response to an acute rise in temperatures has been reported in mitochondria from different ectothermic tissues (Abele et al., 2002; Heise et al., 2003; Paital and Chainy, 2014). Nevertheless, it must be kept in mind that **Figure 6** only summarized the patterns observed at the level of the liver mitochondria. Although these results might preclude alteration in the liver energy homeostasis in a warm environment, they must be taken with caution when attempting to extrapolate to whole cells and organisms. Firstly, the cell has a supplementary antioxidant capacity which can handle H_2O_2 efflux from the mitochondria and thus limit oxidative stress over a larger range of temperatures than the isolated mitochondria (Iftikar and Hickey, 2013). Second, the animal may adjust its behavior or physiology by reducing its activity or even undergoing metabolic depression (e.g., aestivation) when environmental resources become limited in order to alleviate the thermal change in ATP production efficiency (Bishop and Brand, 2000; Kayes et al., 2009).

CONCLUSION

All of these results suggest that *B. bufo* would have to allocate extra energy to maintain ATP production and liver metabolism and to alleviate oxidative stress in order to survive an extremely hot-weather event. In addition, high temperatures will also induce behavioral adjustments that often result to restricting activity and foraging time (Kearney et al., 2009) or restricting space usage (Sears et al., 2016). Such lower capacity to obtain food together with the accelerating metabolic costs with a lower allocation of ATP to performances might lead to a "metabolic meltdown" of ectotherms for the next decades (Huey and Kingsolver, 2019). Nevertheless, the thermal decrease in coupling efficiency (ATP/O) and increase in ROS release can also trigger specific intracellular signaling pathways, which would contribute to the cellular energy metabolism adaptation and subsequent normalization of the initial intracellular perturbation (Seebacher et al., 2010; Ristow and Schmeisser, 2011; Zhang et al., 2018). Such cellular responses may promote long-term resistance to oxidative or metabolic challenges, ultimately contributing to the emergence of new phenotypes (Marrot et al., 2017).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Prefecture du Rhône and the French Ministry of Agriculture (DSV permit no. 692661232).

AUTHOR CONTRIBUTIONS

DR and YV contributed to the conception of the study and the design of the work, the validation and statistic of data, and the writing and editing of the manuscript. DR took charge of the acquisition and analysis of data.

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REVIEW
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Variable Climates Lead to Varying Phenotypes: "Weird" Mammalian Torpor and Lessons From Non-Holarctic Species

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Mammalian heterotherms, species that employ short or long periods of torpor, are found in many different climatic regions. Although the underlying physiological mechanisms of heterothermy in species from lower latitudes (i.e., the tropics and southern hemisphere) appear analogous to those of temperate and arctic heterotherms, the ultimate triggers and resulting patterns of energy expenditure and body temperature are often noticeably different. Phenotypic flexibility in the patterns of thermoregulation in non-Holarctic species can be extensive (depending on body condition, environmental parameters and species competition) and the factors responsible for inducing heterothermy are more variable in non-Holarctic species. As well as being a regular adaptation to seasonality, heterothermy can also be employed as a response to unpredictability in environmental parameters and as a response to emergency situations. Non-Holarctic heterotherms also challenge the notion that regular inter-bout arousals during hibernation are obligatory and suggest all that is necessary to maintain proper functioning during hibernation is an occasional passive return to—or maintenance of—a relatively high body temperature. The study of non-Holarctic heterotherms has led to the conclusion that heterothermy must be defined on the basis of mechanistic, physiological parameters, and not solely by body temperature; yet we are still limited in our abilities to record such mechanistic parameters in the field. It is now believed that homeothermy in mammals evolved in hot climates via an ancestral heterothermic state. Similar to extant warm-climate heterotherms, early mammals could have relied mainly on passive body temperature regulation with a capacity for short- to longer-term up-regulation of metabolism when needed. Hibernation, as seen in temperate and arctic species may then be a derived state of this ancestral heterothermy, and the study of torpor in warm climates can provide potential models for the energetics of early mammals.

Keywords: hibernation, heterothermy, southern hemisphere, tropics, torpor

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INTRODUCTION

Torpor in heterothermic endotherms is a controlled, reversible depression of metabolic rate, and active thermoregulation, well below the usual daily cycle (sensu Geiser and Ruf, 1995). Over the last two to three decades, it has become apparent that torpid states in endotherms are employed in a wide range of ecological and physiological settings and under contrasting conditions (Cossins and Barnes, 1996; Geiser and Brigham, 2012; Boyles et al., 2013; Levesque et al., 2016; Nowack et al., 2017). This challenges the “traditional” view of torpor as essentially an adaptation to mismatches between energy supply and demand during cold seasonal northern hemisphere winters (Geiser, 2004b; Heldmaier et al., 2004; McKechnie and Mzilikazi, 2011). Understanding the different underlying causes and initiators of heterothermy is essential if we are to understand how homeothermy evolved (Grigg and Beard, 2000; Lovegrove, 2012). Torpor use in temperate and arctic species is traditionally differentiated into “daily torpor” and “long-term hibernation.” Daily torpor consists of hypometabolic phases of less than 24 h, with regular, euthermic behavior during the usual active phase, whereas hibernation consists of a series of hypometabolic phases (multiday torpor bouts) over several months that are interspersed with regular euthermic arousals. In “classic” hibernators periodic, or inter-bout, arousals occur about once every 1 or 2 weeks and are presumably needed to sustain mammalian life during long-term torpor. Importantly, however, arousals are energetically expensive phases of active rewarming during hibernation (Carey et al., 2003; Murray et al., 2003) and are associated with increased production of reactive oxygen species and cellular damage (Carey et al., 2000; Brown and Staples, 2011; Nowack et al., 2019). Energy savings during hibernation are more pronounced than during daily torpor, but in contrast to daily torpor, hibernation usually requires preparation (e.g., accumulation of fat stores, modifications of the reproductive and digestive system Barnes et al., 1986; Hume et al., 2002; Sheriff et al., 2013 (but see Liu and Karasov (2011) for an example of a subtropical bat species without pre-fattening) and reliably favorable conditions to allow for a quick recovery when animals terminate hibernation with a reduced body condition. Despite the differentiation between daily heterotherms and hibernators, the extent to which species use torpor can vary substantially as we will detail below.

A Global Perspective of Torpor in Mammals: A Continuum of Physiological Responses

Extant non-Holarctic heterotherms, often living in warm, tropical or subtropical climates, are also capable of conserving energy by reducing metabolism. Although temperate and arctic heterotherms usually employ torpor in a seasonal manner to escape unfavorable winter conditions, the responses reported to date for non-Holarctic heterotherms fall on a continuum (Boyles et al., 2013; Levesque et al., 2016). One extreme is represented by largely heterothermic species that have a highly labile body temperature (T_b; i.e., large daily fluctuations of T_b without actively depressed metabolic rate) and employ torpor (large daily amplitudes in T_b and depressed metabolic rate) at any time of the year, with longer bouts during the hibernation season (Grigg and Beard, 2000; Turbill et al., 2003; Lovegrove and Génin, 2008; Levesque and Lovegrove, 2014; Lovegrove et al., 2014a,b; Dausmann et al., submitted). At the opposite end of the continuum are species that are physiologically able to exhibit torpor but do so only rarely (and usually in form of short bouts of torpor) in emergencies when immediate survival is at risk (Nowack et al., 2010; Dausmann et al., 2012). An intermediate form between heterothermy on a very frequent basis throughout the year or only rare torpor use under extreme circumstances, would be the “classical,” seasonal use of torpor. In this case, T_b is kept rather constant outside of the hibernation season and animals show a regular adjustment of energy balance to seasonal unfavorable conditions (such as low temperature, high rainfall, low food availability, see **Table 1**).

The number and phylogenetic diversity of non-Holarctic species with documented torpor use is steadily increasing, with the number doubling in some taxonomic orders since 2011 (**Table 1**, **Figures 1A,B**) (McKechnie and Mzilikazi, 2011). Also, thanks to technological advancements, more physiological data can be obtained in the field, enabling functional insights into heterothermic responses of free-ranging animals with their full physiological potential (Chmura et al., 2018). The purpose of our review is to summarize what is known about torpor use in mammals living outside the Holarctic, including what is traditionally termed sub-tropics and tropics, and the more temperate zones of the southern hemisphere (**Figure 1A**). Therefore, we have synthesized information about the occurrence, the length, minimum T_b and metabolic rate during torpor, as well as the ultimate triggers of torpor use (season, weather, etc.) in heterothermic species outside of the north temperate and arctic zones. We used data from the comprehensive review by Ruf and Geiser (2015) with the addition of more recent descriptions of torpor use in non-Holarctic heterotherms (**Table 1**, **Figures 1A,B**). We focus on the proximate factors influencing torpor use, as well as the physiological similarities and differences between mammals using torpor in diverse habitats. Following on previous syntheses (Grigg, 2004; Grigg et al., 2004; Lovegrove, 2012), we further discuss how knowledge of these proximate, and the ultimate causes of torpor use in extant tropical heterotherms can provide insight into the ancestral mammal condition.

DO NON-HOLARCTIC HETEROTHERMS FALL INTO THE DAILY-HETEROTHERM—HIBERNATOR DICHOTOMY?

Daily torpor and hibernation patterns in non-Holarctic species are similar to those observed in temperate/arctic animals, albeit with some distinctive differences. As far as it is known, the physiological basis seems analogous: metabolism (and other physiological variables, such as heart rate, respiration rate, etc.) is actively depressed to a fraction of euthermic levels, usually during the daily resting phase or at the end of the activity phase,

TABLE 1 | Non-Holarctic heterotherms, including species distribution, patterns of heterothermy and potential factors inducing torpor where known.

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Afrosoricida										
Chrysochloridae										
Amblysomus hottentotus	Hottentot golden mole	Africa	Sub/-tropical	71	SB/HIB	8.6	NA	Field	Scantlebury et al., 2008	Opportunistic, not triggered by temperature, HIB in spring and summer
Eremitalpa granti	Namib desert golden mole	Africa	Sub/-tropical	26	DT	19.3	0.12^F	T_b: Field, TMR: LabW	Fielden et al., 1990	n.d.
Macroscelidae										
Elephantulus myurus	Eastern rock elephant shrew	Africa	Sub/-tropical	57	SB/PT	7.5	0.078	LabW	Lovegrove et al., 2001; Mzilikazi et al., 2002	Torpor in summer and winter; triggered by cold and food reduction
Elephantulus rupestris	Western rock elephant shrew	Africa	Sub/-tropical	53–61	DT	11.9	NA	Field	Oelkrug et al., 2012	Torpor in summer and winter; triggered by cold and high air humidity
Elephantulus edwardii	Cape rock elephant shrew	Africa	Subtropical/ temperate	42–48	SB/PT	9.2	NA	LabW	Geiser and Mzilikazi, 2011	n.d.
Macroscelides proboscideus	Round-eared elephant shrew	Africa	Sub/-tropical	46	DT	9.4	NA	LabW	Lovegrove et al., 1999	Only when food deprived
Tenrecidae										
Echinops telfairi	Lesser hedgehog tenrec	Madagascar	Subtropical	132	SB/PT	12.5	0.026	Lab	Lovegrove and Génin, 2008; Wein, 2010	Year-round, independent of season, temperature and food supply
Geogale aurita	Large-eared tenrec	Madagascar	Subtropical	7	n.d.	24.9	0.15	Lab	Gould and Eisenberg, 1966	n.d.
Hemicentetes nigriceps	Highland streaked tenrec	Madagascar	Subtropical	102	HIB?	NA	NA	LabW	Gould and Eisenberg, 1966; Stephenson and Racey, 1994	n.d.
Hemicentetes semispinosus	Lowland streaked tenrec	Madagascar	Subtropical	133	HIB	22	NA	LabW	Gould and Eisenberg, 1966; Stephenson and Racey, 1994	n.d.
Microgale dobsoni	Dobson's shrew tenrec	Madagascar	Subtropical	45	n.d.	24.2	0.22	LabW	Stephenson and Racey, 1993	n.d.
Microgale talazaci	Talazac's shrew tenrec	Madagascar	Subtropical	44	n.d.	27.4	NA	LabW	Stephenson and Racey, 1993	n.d.

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Setifer setosus	Greater hedgehog tenrec	Madagascar	Subtropical	270	SB/HIB	16.5	0.07	T_b: Field, TMR:LabW	Levesque et al., 2013, 2014; Lovegrove et al., 2014a	SB: triggered by cold year-round; HIB: triggered by seasonal dry/food shortage
Tenrec ecaudatus	Tailless tenrec	Madagascar	Subtropical	1500–2500	SB/HIB	16.5	0.027	LabW	Gould and Eisenberg, 1966; Nicoll, 1986 reviewed in Geiser (2004b), Lovegrove et al. (2014b), Treat et al. (2018)	SB: triggered by cold year-round; HIB: circannual rhythms
Carnivora										
Hyaenidae										
Proteles cristata	Aardwolf	Africa	Sub-/tropical	8000–14000	DT	31.2	NA	Field	Anderson, 2004	Possibly water availability
Chiroptera										
Emballonuridae										
Peropteryx macrotis	Dog-faced bat	America	Tropical	5	n.d.	24.2	1.13^F	LabW	Genoud et al., 1990	n.d.
Taphozous australis	Coastal sheath-tail bat	Australia	Tropical	23	n.d.	16	NA	LabW	Kulzer et al., 1970	n.d.
Taphozous melanopogon	Tomb bat	Asia	Tropical	26	n.d.	27	NA	Lab	Kulzer, 1965	n.d.
Hipposideridae										
Hipposideros terasensis	Formosan Leaf-nosed Bat	Asia	Subtropical	58	HIB	13.8	0.046	LabW	Liu and Karasov, 2011, 2012	HIB in winter; triggers: n.d.
Macronycteris commersoni	Commerson's Leaf-nosed Bat	Madagascar	Sub-/tropical	54	SB, PT, HIB	27.2	0.026	Field	Reher et al., 2018	HIB and PT in winter, PT and DT in summer, triggers: n.d.
Rhinonicteris aurantia	Orange leaf-nosed bat	Australia	Tropical	7	n.d.	23.6	NA	LabW	Kulzer et al., 1970	n.d.
Megadermidae										
Macroderma gigas	Ghost bat	Australia	Tropical	100	n.d.	32	NA	Lab	Geiser et al., unpub. Data in: Geiser and Stawski, 2011	n.d.
Megaderma lyra	Asian false vampire bat	Asia	Tropical	26	n.d.	30	NA	Lab	Kulzer, 1965	Trigger: n.d. but torpor at high ambient temperatures

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Molossidae										
Molossus molossus	Pallas' mastiff bats	South America, North America	Tropical	10–12	DT	25.3	0.519	T_b: Field; TMR: LabW	O'Mara et al., 2017	Food availability
Mormopterus loriae	Little northern freetail bat	Australia	Tropical	9	n.d.	10	NA	LabW	Kulzer et al., 1970	n.d.
Ozimops petersi (in paper still *Mormopterus* species 3)	Inland free-tailed bat	Australia	Subtropical	9	SB/PT	21.3	NA	Field	Bondarenco et al., 2013	Torpor in summer; water saving?
Tadarida aegyptiaca	Egyptian free-tailed bat	Africa	Sub/-tropical	16	SB/PT	6.2	NA	Field	Cory Toussaint et al., 2010	PT at lower ambient temperatures
Tadarida brasiliensis	Brazilian free-tailed bat	Central and South America	Sub/-tropical	11	DT	$\sim15^F$	0.06	LabW	Soriano et al., 2002	Cold (facultative)
Tadarida condylurus (formerly Mops condylurus)	Angolan free-tailed bat	Africa	Sub/-tropical	28–34	DT	12	NA	Field	Maloney et al., 1999; Vivier and van der Merwe, 2007	Year-round use of torpor triggered by food reduction and cold
Tadarida teniotis	European free-tailed Bat	Africa, Asia, Europe	Subtropical	32	n.d.	16–17	0.04^F	LabW	Marom et al., 2006	Cold
Natalidae										
Natalus tumidirostris	Funnel-eared bat	America	Tropical	5	n.d.	23.6	0.67^F	LabW	Genoud et al., 1990	n.d.
Nycteridae										
Nycteris thebaica	Egyptian slit-faced bat	Africa	Sub/-tropical	11	n.d.	28.4	NA	LabW	Unpublished data, Cory Toussaint, McKechnie, Brigham in: McKechnie and Mzilikazi, 2011; Cory Toussaint et al., 2013	n.d.; No torpor use found in wild
Phyllostomidae										
Carollia perspicillata	Leaf-nosed bat	America	Sub/-tropical	20	n.d.	22	1.01	LabW	Audet and Thomas, 1997	Food restriction
Glossophaga soricina	Long-tongued bat	America	Sub/-tropical	10	DT	21	0.23	Lab	Kelm and Helversen, 2007	Food restriction
Sturnira erythromos	Hairy yellow-shouldered bat	South America	Tropical	16	DT	$\sim15^F$	0.2^F	LabW	Soriano et al., 2002	Cold (facultative)

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO_2 h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Sturnira lilium	Yellow-shouldered bat	America	Sub/-tropical	17	n.d.	23	0.5	LabW	Audet and Thomas, 1997	Food restriction
Vampyrops (Platyrrhinus) helleri	Heller's broad-nosed bat	America	Sub/-tropical	15	n.d.	28.5	NA	Lab	Rasweiler, 1973	Food deprivation
Pteropodidae										
Dobsonia minor	Bare-backed fruit bat	Asia	Tropical	74	n.d.	26	0.75[F]	LabW	McNab and Bonaccorso, 2001	n.d.
Macroglossus minimus	Northern blossom-bat	Asia, Australia	Tropical	16	DT	23.1	0.52	LabW	Bartels et al., 1998	Cold
Megaloglossus woermanni	Long-tongued fruit bat	Africa	Tropical	12	n.d.	26.2	0.80	Lab	Kulzer and Storf, 1980	n.d.
Nyctimene albiventer	Tube-nosed bat	Asia	Tropical	28	DT	25.5	0.67	LabW	Bartholomew et al., 1970	n.d.
Nyctimene robinsoni	Eastern tube nosed bat	Australia	Sub/-tropical	50	n.d.	n.d.	NA	Lab	Hall and Pettigrew, 1995	n.d.
Syconycteris australis	Common blossom bat	Australia	Subtropical	18	DT	17.2	0.47	Lab	Coburn and Geiser, 1998	Food and water deprivation; longer and deeper bouts in summer
Rhinolophidae										
Rhinolophus megaphyllus	Eastern horseshoe bat	Asia, Australia	Sub/-tropical	8	n.d.	16	NA	LabW	Kulzer et al., 1970	n.d.
Rhinopomatidae										
Rhinopoma cystops	Egyptian mouse-tailed bat	Africa, Asia	Sub/-tropical	12	HIB	1	0.16	Field (T_b) and LabW (MR)	Levin et al., 2012, 2015	HIB in winter; cold, or food shortages
Rhinopoma microphyllum	Greater mouse-tailed bat	Africa, Asia	Sub/-tropical	25	SB/HIB	21[F]	0.14	LabW	Kulzer, 1965; Levin et al., 2015	SB? for males and non-lactating females in summer, HIB in winter. Cold or food shortages during winter
Vespertilionidae										
Chalinolobus gouldii	Gould's wattled bat	Australia	Wide distribution, tropical	17.5	n.d.	12	0.05	Lab	Hosken and Withers, 1997	n.d.
Chalinolobus morio	Chocolate wattled bats	Australia	Subtropical/ temperate	8.1	SB/PT/HIB	3.4	NA	Field	Turbill, 2006	SB? in summer, PT (males) and HIB (females) in winter; trigger: cold

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Lasiurus cinereus	Hoary bat	America	Wide distribution, tropical	33	SB/PT*	2	0.37	LabW	Cryan and Wolf, 2003; Willis et al., 2006	Reproduction, storm
Myotis adversus	Large-footed myotis	Asia	Tropical	8	PT	8	NA	LabW	Kulzer et al., 1970	n.d.
Miniopterus schreibersii	Large bentwing/Schreiber's long-fingered bat	Africa	Tropical	15	n.d.	5	0.24	LabW	Brown (1999)	Torpor in summer and winter, trigger: n.d.
Neoromicia (Pipistrellus) tenuipinnis	White-winged serotine	Africa	Tropical	5	HIB	n.d.	NA	Anecdotal field evidence	Eisentraut (1956)	n.d.
Nyctophilus bifax	Northern long-eared bat	Australia	Tropical	9	SB/PT	9.4	0.046	LabW	Stawski et al., 2009	SB? in summer, PT during winter; triggered by cold, weather condition
Nyctophilus geoffroyi	Lesser long-eared bat	Australia	Wide distribution, tropical	8	SB/HIB	1.4	0.037	LabW	Geiser and Brigham, 2000; Turbill and Geiser, 2008; Geiser et al., 2011	Seasonal, independent of ambient temperature
Nyctophilus gouldi	Gould's long-eared bat	Australia, Asia	Wide distribution, tropical	10	SB/HIB	2.3	0.052	LabW	Geiser and Brigham, 2000; Turbill and Geiser, 2008	HIB in winter, SB?during the rest of the year Trigger: cold
Otonycteris hemprichii	Desert long-eared bat	Africa, Asia	Subtropical	26	n.d.	20–23	0.209^F	LabW	Marom et al., 2006	Cold
Scotophilus dinganii	African yellow bat	Africa	Sub/-tropical	29	DT	18.5	NA	Field	Jacobs et al., 2007	n.d.
Scotophilus mhlanganii	recently described	Africa	Sub/-tropical	28	DT	18.2	NA	Field	Jacobs et al., 2007	n.d.
Scotorepens balstoni	Inland broad-nosed bat	Australia	Subtropical	10	DT	15.1	0.044	Field	Geiser and Brigham, 2000; Bondarenco et al., 2016	Torpor in summer; Trigger: n.d.
Scotorepens greyii	Little broad-nosed bat	Australia	Subtropical	6	DT	15.3	NA	Field	Bondarenco et al., 2016	Torpor in summer; Trigger: n.d.
Vespadelus vulturnus	Little forest bat	Australia	Subtropical	4	DT	5	0.014	LabW	Willis et al., 2005	Food withdrawal
Cingulata										
Chlamyphoridae										
Zaedyus pichiy	Dwarf armadillo	America	Subtropical/ temperate	700–1500	SB/HIB	12.5	NA	Lab	Superina and Boily, 2007	n.d.
Eulipothyphla										
Erinaceidae										
Atelerix frontalis	Southern African hedgehog	Africa	Sub/-tropical	394–797	SB/HIB	1	NA	Field	Hallam and Mzilikazi, 2011	Seasonal, individuals with high body mass hibernate

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Sorcidae										
Crocidura flavescens	Greater red musk shrew	Africa	Temperate	31.5	DT	n.d.	NA	LabW	Baxter, 1996	n.d.
Marsupialia										
Dasyuromorphia										
Dasyuridae										
Antechinomys laniger	Kultarr	Australia	Wide distribution, tropical	27.4	DT	11	0.14	Lab	Geiser, 1986	Spontaneous torpor at low temperature; torpor induced by food withdrawal at higher temperature.
Antechinus flavipes	Yellow-footed antechinus	Australia	Wide distribution, tropical	30–70	DT	17.8	0.48	LabW	Geiser, 1988; Rojas et al., 2014	Body mass effects torpor stronger than season, frequency increases with age, torpor in response to fires
Antechinus stuartii	Brown antechinus	Australia	Subtropical/ temperate	26	DT	18.1	0.66	Field (T_b) and LabW (MR)	Geiser, 1988; Hume et al., 2019	Torpor in response to fires
Dasycercus blythi	Brush-tailed mulgara	Australia	Sub/-tropical	70	DT	10.8	NA	Field	Körtner et al., 2008, 2016	Independent of resource availability; linked to reproduction
Dasycercus cristicauda	Crest-tailed mulgara	Australia	Sub/-tropical	100	DT	13.2	0.12	LabW	Geiser and Masters, 1994; Körtner et al., 2016	Independent of resource availability; linked to reproduction
Dasykaluta rosamondae	Little red kaluta	Australia	Semi-arid	35.5	DT	18^{F}	0.33	Lab	Withers and Cooper, 2009	Food withdrawal
Dasyuroides byrnei	Kowari	Australia	Tropical/subtropical	120	DT	20.4	0.4	Lab	Geiser and Baudinette, 1987	Spontaneous DT, trigger: cold.
Dasyurus geoffroii	Western quoll	Australia	Subtropical/ temperate	1000	DT	23.1	NA	Not known	Arnold, 1976	n.d.
Dasyurus hallucatus	Northern quoll	Australia	Tropical	516	DT	28.4	NA	LabW	Cooper and Withers, 2010	n.d.
Dasyurus viverrinus	Eastern quoll	Australia	Temperate	1000	DT	25	NA	Not known	Moyle, 1999	n.d.
Myrmecobius fasciatus	Numbat	Australia	Subtropical/ temperate	n.d.	DT	19.1	NA	Field	Cooper and Withers, 2004	Spontaneous DT, in winter more frequent, deeper and longer; trigger: cold
Ningaui yvonneae	Southern ningaui	Australia	Subtropical/ temperate	11.6	DT	15.3	0.3	Lab	Geiser and Baudinette, 1988	Spontaneous DT, trigger: cold. Frequency increased with food withdrawal

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Planigale gilesi	Giles' planigale	Australia	Subtropical	8.3	DT	14.3	0.36	Lab	Geiser and Baudinette, 1988	Spontaneous DT, trigger: cold. Frequency increased with food withdrawal
Planigale ingrami	Long-tailed planigale	Australia	Tropical	6–9	DT	n.d.	0.48	Lab	Dawson and Wolfers, 1978	n.d.
Planigale maculata	Common planigale	Australia	Tropical	10–16	DT	19.6	0.4	Lab	Morton and Lee, 1978	Triggered by cold, independent of food availability
Planigale tenuirostris	Narrow-nosed planigale	Australia	Wide distribution/tropical	6.6–7.3	DT	n.d.	0.48	LabW	Dawson and Wolfers, 1978	n.d.
Pseudantechinus macdonnellensis	Fat-tailed false antechinus	Australia	Arid-zone	18–33	DT	15.7	NA	Field	Geiser and Pavey, 2007	n.d.
Sminthopsis crassicautata	Fat-tailed dunnart	Australia	Arid-zone	10	DT	10.8	0.27	Lab	Geiser and Baudinette, 1987; Warnecke et al., 2008	n.d.
Sminthopsis douglasi	Julia Creek dunnart	Australia	Tropical	60	DT	16.9	0.43	not known	Muller, 1996	n.d.
Sminthopsis macroura	Stripe-faced dunnart	Australia	Wide distribution, tropical	20–28	DT	11.3	0.3	Lab	Geiser and Baudinette, 1987; Song et al., 1998; Körtner and Geiser, 2008	Independent of season; triggered by temperature and food/water restriction; deeper at lower temperatures and without food
Sminthopsis murina	Common dunnart	Australia	Wide distribution, tropical	18	DT	15	0.25	Lab	Geiser et al., 1984	n.d.
Sminthopsis ooldea	Ooldea dunnart	Australia	Arid-zone	11	DT	n.d.	0.77	LabW	Tomlinson et al., 2012	n.d.
Microbiotheriidae										
Dromiciops gliroides	Monito del monte	America	Subtropical/ temperate	38.9	SB/PT	7.1	0.03	LabW	Grant and Temple-Smith, 1987; Nespolo et al., 2010	Ambient temperature; food availability, but torpor use even when food available
Didelphimorphia										
Didelphidae										
Gracilinanus agilis	Agile gracile opossum	America	Tropical	12–43.6	DT	20	0.3	LabW	Cooper et al., 2009	Lab study only. Capable of torpor at high ambient temperatures

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Gracilinanus microtarsus	Brazilian gracile opossum	America	Tropical	13	DT	16	NA	LabW	Morrison and McNab, 1962	Lab study only (*n* = 1). Capable of torpor at high ambient temperatures
Lestodelphys halli	Patagonian opossum	America	Subtropical/temperate	48	SB/PT*	7.7	NA	LabW	Geiser and Martin, 2013	Cold exposure, food withdrawal, but also torpor use when food available
Marmosa robinsoni	Robinson's mouse opossum	America	Tropical	122	DT?	23	NA	LabW	McNab, 1978	n.d.
Monodelphis brevicaudata	Red-legged short-tailed opossum	America	Tropical	40–111	DT?	27	NA	LabW	McNab, 1978	n.d.
Monodelphis domestica	Gray short-tailed opossum	America	Tropical	93	DT	28.6	0.365	Lab	Busse et al., 2014	n.d. but enters torpor at high ambient temperatures
Thylamys elegans	Elegant fat-tailed opossum	America	Wide distribution, tropical	32.1	DT	11^F	0.4	LabW	Opazo et al., 1999; Bozinovic et al., 2007	Food reduction
Diprotodontia										
Acrobatidae										
Acrobates pygmaeus	Feathertail glider	Australia	Wide distribution, tropical	14	SB/PT	2	0.042	LabW	Jones and Geiser, 1992; Geiser and Ferguson, 2001	PT in emergency situations
Burramyidae										
Burramys parvus	Mountain pygmy possums	Australia	Temperate	40	HIB	1.8	0.025	LabW	Geiser and Broome, 1991	Seasonal
Cercartetus caudatus	Long-tailed pygmy possum	Australia	Tropical	30	SB/HIB?	n.d.	NA	Lab	Atherton and Haffenden, 1982	n.d.
Cercartetus concinnus	Western pygmy possum	Australia	Temperate/semi-arid	18	HIB	4.7	0.046	Lab	Geiser, 1987	n.d.
Cercartetus lepidus	Tasmanian pygmy possum	Australia	Temperate	12	SB/PT*	5.9	0.052	Lab	Geiser, 1987	n.d.
Cercartetus nanus	Eastern pygmy possum	Australia	Subtropical	20	SB/HIB	1.3	0.018	LabW	Geiser, 1993; Song et al., 1997; Turner et al., 2012	Food withdrawal; opportunistic
Petauridae										
Petaurus breviceps	Sugar glider	Australia	Wide distribution, tropical	130	DT	10.4	0.03	Field (T_b) and LabW (MR)	Fleming, 1980; Körtner and Geiser, 2000; Christian and Geiser, 2007	Reluctant to enter torpor; triggered by rain and cold

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Tarsipedidae										
Tarsipes rostratus	Honey possum	Australia	Wide distribution	5–15	DT	5.4	0.15	LabW	Collins et al., 1987	Triggered by food shortage and low temperature
Monotremata										
Tachyglossidae										
Tachyglossus aculeatus	Short-beaked echidna	Australia	Wide distribution, tropical	2800	SB/HIB/PT	4	0.03	Field (T_b) and LabW (MR)	Augee and Ealy, 1968; Grigg et al., 1989	Seasonal HIB year-round use of SB?-food withdrawal, cold
PRIMATES										
Lemuridae										
Allocebus trichotis	Hairy-eared dwarf lemur	Madagascar	Tropical	70	SB/HIB?	n.d.	NA	Anecdotal field evidence	Dausmann, 2014	n.d.
Cheirogaleus crossleyi	Furry-eared dwarf lemur	Madagascar	Tropical	350	HIB	9	NA	Field	Blanco and Rahalinarivo, 2010	Seasonal
Cheirogaleus major	Greater dwarf lemur	Madagascar	Tropical	300	HIB	n.d.	NA	Field	Lahann, 2007	Seasonal
Cheirogaleus medius	Fat-tailed dwarf lemur	Madagascar	Tropical	130	HIB	9.3	0.044	Field	Dausmann et al., 2005	Seasonal
Cheirogaleus sibreei	Sibree's dwarf lemur	Madagascar	Tropical	250	HIB	n.d.	NA	Field	Blanco et al., 2013	Seasonal
Microcebus berthae	Madame Berthe's mouse lemur	Madagascar	Tropical	31	DT	6.8	0.09	Field	Ortmann et al., 1997; Schmid et al., 2000	Seasonal
Microcebus griseorufus	Reddish-gray mouse lemur	Madagascar	Tropical	50	SB/PT/HIB	6.5	0.15	Field	Kobbe and Dausmann, 2009; Kobbe et al., 2011, 2014	Seasonal, hibernation depending on body mass
Microcebus murinus	Gray mouse lemur	Madagascar	Tropical	70	SB/PT?/HIB	7.8	0.16	Field	Schmid, 2000; Schmid and Speakman, 2000	Seasonal, hibernation depending on body mass and sex
Microcebus ravelobensis	Golden-brown mouse lemur	Madagascar	Tropical	63	DT	25^F	NA	Field	Lovegrove et al., 2014a	Seasonal
Microcebus rufus	Brown mouse lemur	Madagascar	Tropical	40	SB/PT?/HIB?	n.d.	NA	Field	Atsalis, 1999; Randrianambinina et al., 2003	Seasonal
Mirza coquereli	Coquerel's mouse lemur	Madagascar	Tropical	n.d.	DT	n.d.	NA	Anecdotal field evidence	Dausmann, 2008	Seasonal

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Lorisidae										
Galago moholi	African lesser bushbaby	Africa	Sub/-tropical	100	DT	13.5	0.09	Field	Nowack et al., 2010, 2013b	Emergency, low body mass, food withdrawal
Loris tardigradus tardigradus	Red slender loris	Asia	Tropical	85–369	DT	n.d.	NA	Field	pers. obs. KAI Nekaris	n.d.
Nycticebus javanicus	Javan slow loris	Asia	Tropical	600	DT	n.d.	NA	Anecdotal field evidence	pers. obs. KAI Nekaris	n.d.
Nycticebus pygmaeus	Pygmy slow loris	Asia	Tropical	450	PT	11	NA	LabW	Streicher, 2004; Ruf et al., 2015	Seasonal
RODENTIA										
Bathergidae										
Cryptomys hottentotus darlingi	Mashona mole rat	Africa	Sub/-tropical	60	DT	26.8	NA	Lab	Bennett et al., 1993	Cold
Fukomys damarensis	Damaraland mole rat	Africa	Sub/-tropical	88–202	n.d.	28.5	NA	Lab	Streicher, 2010; Boyles et al., 2012	Seasonal?
Cricetidae										
Calomys musculinus	Drylands vesper mouse	America	Sub/-tropical	20	DT	28	0.52	LabW	Bozinovic and Rosenmann, 1988	Aridity? Spontaneous torpor when food supplied
Calomys venustus	Córdoba vesper mouse	America	Sub/-tropical	49.5	DT	16.4	0.96	LabW	Caviedes-Vidal et al., 1990	n.d.
Phyllotis darwini	Darwin's leaf-eared mouse	America	Temperate	35.9	DT	17.5	0.19	LabW	Bozinovic and Marquet, 1991	Food withdrawal
Muridae										
Acomys russatus	Golden spiny mouse	Africa/Middle East	Sub/-tropical	50–72	SB/HIB	24	0.25	Lab	Grimpo et al., 2013; Barak et al., 2019	Torpor use at high temperature and increased during food restriction
Aethomys namaquensis	Namaqua rock mouse	Africa	Sub/-tropical	46	n.d.	19.8	NA	Field	Withers et al., 1980	n.d.
Gerbillus pusillus	Least gerbil	Africa	Tropical	13	DT	16.7	0.38	LabW	Buffenstein, 1984	Food deprivation
Mus musculus	House mouse	Worldwide	Wide distribution, tropical	13	DT	20.5	0.7	Lab	Hudson and Scott, 1979; Tomlinson et al., 2007; Schubert et al., 2010	Cold, food deprivation

(Continued)

TABLE 1 | Continued

Species	Common name	Distribution		M_b (g)	DT/PT/HIB	T_{min} (°C)	TMR (mLO$_2$ h^{-1} g^{-1})	Lab/Field	References	Potential torpor triggers
Saccostomus campestris	Pouched mouse	Africa	Sub/-tropical	60–87	DT	21	0.35	Lab	Lovegrove and Raman, 1998; Mzilikazi and Lovegrove, 2002	Year-round, triggered by cold and in females also by food restriction
Steatomys pratensis	Fat mouse	Africa	Tropical	16	DT	13	0.3	Lab	Ellison, 1995	Cold
Petromyscus collinus	Pygmy rock mouse	Africa	Sub-tropical	19	n.d.	15.6	NA	Field	Withers et al., 1980	n.d.
Pseudomys albocinereus	Ash-grey mouse	Australia	Wide distribution, subtropical	28	DT	24.5	1.02	LabW	Barker et al., 2012	Torpor use at 20/25°C. presumably triggered by food withdrawal
Pseudomys hermannsburgensis	Sandy inland mouse	Australia	Wide distribution, tropical	12	DT	17.3	0.85^F	LabW	Tomlinson et al., 2007	Hypothermia in response to cold, no spontaneous arousal
Rattus fuscipes	Bush rat	Australia	Wide distribution, subtropical	119–151	DT	23.8	NA	Field	Nowack and Turbill, unpublished data	Torpor use in response to hindered foraging activity
Gliridae (Myoxidae)										
Graphiurus murinus	Woodland dormouse	Africa	Sub/-tropical	45	PT	1.5	NA	Field	Mzilikazi et al., 2012	n.d.
Graphiurus ocularis	Spectacled dormouse	Africa	Subtropical/ temperate	67.8	n.d.	<31	NA	LabW	Perrin and Ridgard, 1999	n.d.

Species are chosen on basis of Ruf and Geiser (2015); distributions are based on the IUCN distribution maps. HIB, hibernation (several months); PT, prolonged torpor (several days); DT, daily torpor (<24 h); SB, short torpor bouts undergone by hibernators; n.d., no data; M_b, body mass; T_{min}, minimal body temperature during torpor; TMR, torpid metabolic rate, Lab/Field: Field, evidence from field studies or from wild animals (captured from the field and usually measured within 24 h to a few days) in a field laboratory under natural light and temperature conditions; Lab, evidence from laboratory measurements; LabW, evidence from laboratory measurements on wild animals, i.e., captured from the field and usually measured within 24 h to a few days.

**Termed "hibernation" in the publication, but we further differentiate multiday torpor bouts into prolonged torpor and hibernation based on length.*

^{F}*Number estimated from figure.*

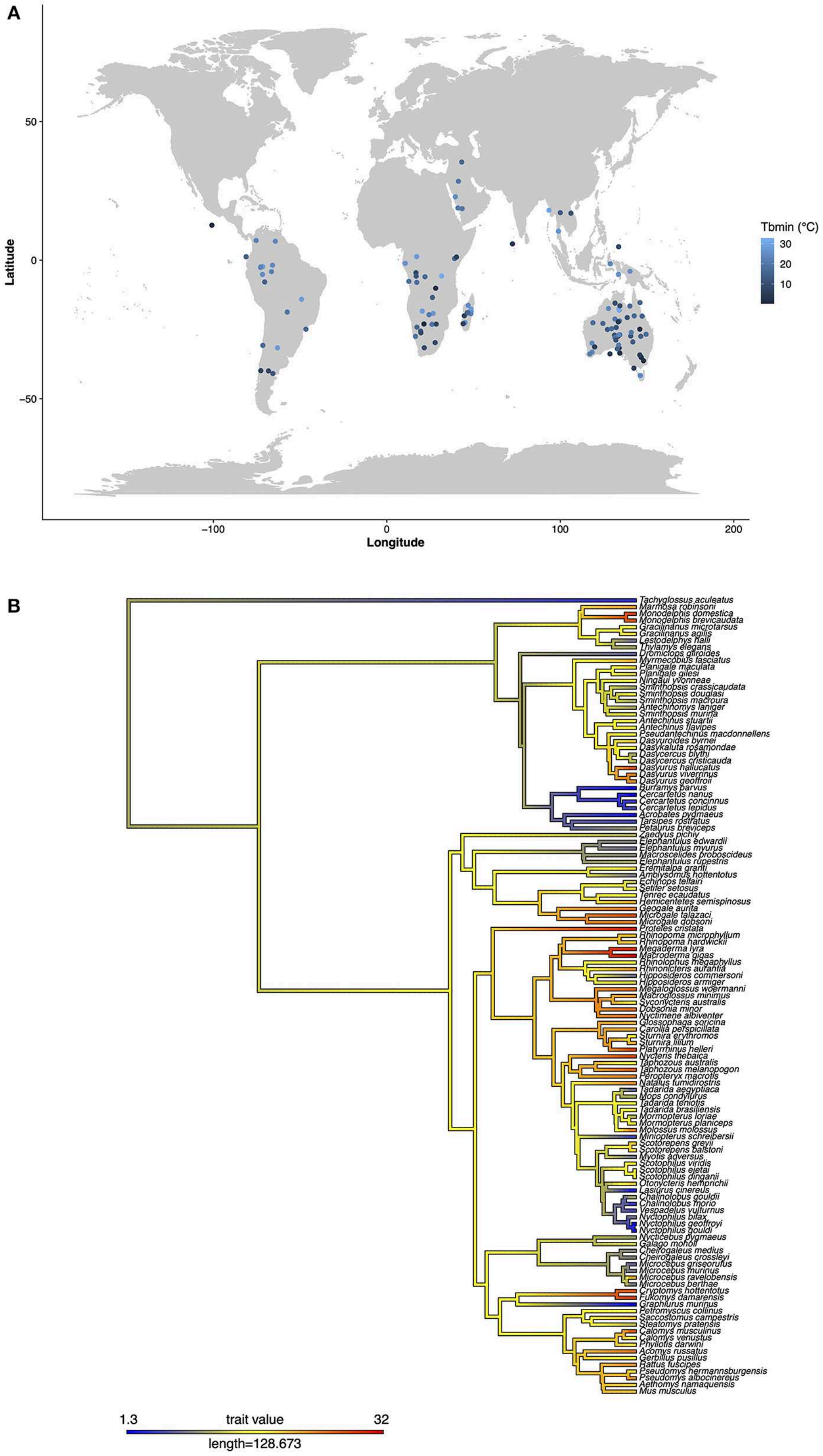

FIGURE 1 | (A) The minimum torpid body temperature ($T_b$min) and phylogenetic relationship of non-Holarctic heterotherms, and **(B)** their geographical distribution. The phylogeny, including estimated ancestral states for $T_b$min was plotted using "contMap" in the R library "phytools" (Revell, 2012) and the latest mammalian phylogeny by Upham et al. (2019). Geographical locations were taken as the latitude and longitude at the middle of the species range provided by the PanTHERIA dataset (Jones et al., 2009).

and T_b adjusts accordingly (Carey et al., 2003; Geiser, 2004a; Heldmaier et al., 2004). Additionally to active depression of metabolic rate, the abandoning of the differential between T_b and ambient temperature contributes substantially to energy savings during torpor bouts, especially in small species. Usually this results in a drop in T_b, the degree of which depends on ambient temperature (and the degree of daily fluctuations thereof), the insulative capacity of the resting site (see below), and the duration of the torpor bout (Hallam and Mzilikazi, 2011; Kobbe et al., 2011).

Although we can still differentiate daily heterotherms and hibernators in the non-Holarctic dataset, the distinction becomes a bit more blurry. Notably, it is more common for non-Holarctic hibernators to also use short bouts of torpor that typically last less than 24 h but during which metabolic rate is lowered to levels comparable to those during hibernation phases, e.g., seen in *Nyctophilus* spp. (Geiser and Brigham, 2000; Geiser and Stawski, 2011). To differentiate these from daily torpor use by daily heterotherms, we will refer to those by using the term "short bouts". Furthermore, many non-Holarctic hibernators do not only use short bouts or hibernation, but often also show an intermediate length of torpor, i.e., "prolonged torpor" lasting up to several days with regular activity (several hours to many days) between torpor bouts (Kobbe et al., 2011; Dausmann, 2014).

HETEROTHERMY AS THE NORM, HOMEOTHERMY AS THE EXCEPTION

Although Holarctic heterotherms usually maintain a high and stable T_b during part of the year, examples from the southern hemisphere show us that this is not the case for all heterotherms. At the extreme end of thermolabililty in heterotherms are the eutherian Tenrecidae, a family of mammals found on Madagascar and the surrounding islands, which have been isolated from the mainstream of mammalian evolution for about 30–56 Myr (Crompton et al., 1978; Everson et al., 2016). They are members of the superorder Afrotheria, a group of mammals whose extant members live predominantly in Africa or are of African origin (Poux et al., 2005). The ecology, behavior and thermoregulatory physiology of these "basoendothermic" mammals have been proposed to be similar to those of the early mammalian endotherms (Lovegrove and Génin, 2008; Levesque and Lovegrove, 2014). All members of this group investigated to date exhibit a generally low basal metabolic rate and highly labile T_b, often closely tracking environmental temperature (**Table 1**). Even so, the lesser hedgehog tenrec, *Echinops telfairi* (and likely other species of tenrec as well), has functional brown adipose tissue, enabling non-shivering thermogenesis (Oelkrug et al., 2013). In addition to low basal metabolic rates, many species of tenrecs also enter short daily bouts of torpor and long-term hibernation (**Table 1**). Some species (*E. telfairi*, *Setifer setosus*) seem to maintain euthermy (higher and less variable T_b in the resting-phase) only during reproduction (gestation and/or lactation) (Poppitt et al., 1994; Wein, 2010; Levesque and Lovegrove, 2014; Lovegrove et al., 2014b) presumably to enhance embryonic development and milk production. For males, the occasional rise of T_b above 30°C might be sufficient to allow sperm production (Fietz et al., 2004).

Outside of the hibernation season (during the austral winter), torpor in *E. telfairi* and *S. setosus* seems to be independent of external stimuli and is employed frequently not in response to seasonality or a scarcity of food or water, but throughout the active season (see **Figure 2**; Lovegrove and Génin, 2008; Wein, 2010; Levesque et al., 2013, 2014; Dausmann et al., submitted). Interestingly, the thermolability shown by many tenrec species is mirrored by another basoendotherm (dubbed "protoendotherm" by Grigg et al., 2004), the short-beaked echidna, *Tachyglossus aculeatus* (Order Monotremata). Echidnas have a wide distribution in Australia and Tasmania, from cold-temperate to desert habitats, and also occur in tropical areas. They exhibit daily fluctuations in T_b (up to 5°C) and use torpor flexibly (Kuchel, 2003; Grigg et al., 2004; Nicol and Andersen, 2006). Depending on their habitat they increase torpor use during the cold period and either show long-term hibernation (e.g., Tasmania or Australian Alps: Augee and Ealy, 1968; Grigg et al., 1989; Nicol and Andersen, 2002) or prolonged torpor lasting for a few days (Brice et al., 2002; Kuchel, 2003; Western Australian Wheatbelt: Nowack et al., 2016).

Although this form of continuous heterothermy, either via torpor or highly variable T_b, has to date predominantly been found in spiny, terrestrial insectivores such as tenrecs and echidnas, it may possibly exist in other groups. For example, another tropical species that uses torpor on a regular day-to-day basis is the Angolan free-tailed bat, *Tadarida aegyptiaca.* This species uses daily torpor throughout the year and continuously maintains its T_b close to ambient temperatures when at rest (Vivier and van der Merwe, 2007). Given the large phylogenetic range covered by these three groups and the small number of studies investigating thermophysiology in the field, it is likely that there are other species of mammals with similar levels of thermolability that will be revealed with further study.

SEASONAL HETEROTHERMIC RESPONSES

Strictly seasonal use of torpor seems to be less common in lower latitude heterotherms than in their northern counterparts (**Table 1**). Furthermore, although most species show regular, seasonal use of daily or prolonged torpor, the use of hibernation, in the classical sense of animals disappearing for months at a time, is not as widespread in non-Holarctic species as it is in temperate/arctic mammalian lineages (Heldmaier et al., 2004; McKechnie and Mzilikazi, 2011; Ruf and Geiser, 2015). In contrast to those tenrec species highlighted above that use some form of heterothermic response during most of the year, other species, such as the tailless tenrec, *Tenrec ecaudatus,* are generally believed to only hibernate seasonally (Nicoll, 1986). Although based on more recent studies, it is highly likely that they can also use short bouts of torpor outside of the regular hibernation period (Lovegrove et al., 2014b; Treat et al., 2018).

Interestingly, the most strictly seasonal hibernators in the tropics are concentrated in one family of small, nocturnal

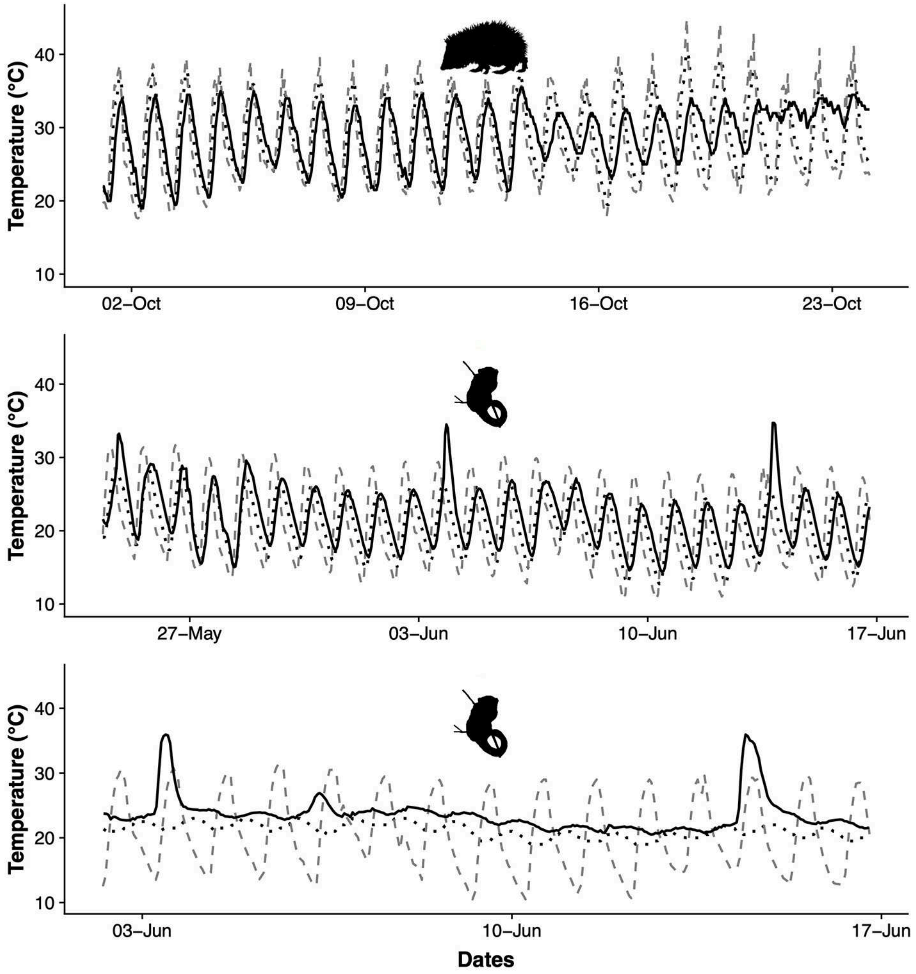

FIGURE 2 | Top panel: Body temperature (black), ambient temperature (dashed gray) and temperature from a nearby nest site (dotted black) of a free ranging greater hedgehog tenrec (*Setifer setosus*) during the Austral winter (dry season). Redrawn from Lovegrove et al. (2014a) and Levesque et al. (2014). Middle and lower panel: Body temperature (black), ambient temperature (dashed gray) and tree-hole temperature (dotted black) of a free ranging fat-tailed dwarf lemur (*Cheirogaleus medius*) during the Austral winter (dry season) in a poorly insulated (middle) or moderately well insulated (lower panel) tree-hole.

Malagasy lemurs, the Cheirogaleidae (**Table 1**). It is likely that all species of this family (i.e., the genera *Allocebus*, *Cheirogaleus*, *Microcebus*, *Mirza*) are heterotherms, with the exception of members of one genus (*Phaner,* which has a very specific feeding regime that does not change seasonally) and most species of this family studied to date become torpid during the austral winter under free-ranging conditions (Dausmann, 2008, 2014). Thus, heterothermy in lemurs is a seasonal response, decreasing energy and water demands drastically thereby facilitating survival during the harsh (cold and dry) conditions of the Malagasy winter (Schmid and Speakman, 2000; Schmid et al., 2000). During the dry season in winter, temperatures decline across all habitats of Madagascar, although the extent varies. All dwarf lemurs (*Cheirogaleus*) are obligate hibernators in their natural environments (Petter, 1978; Hladik et al., 1980; Dausmann, 2008, 2014), from the eastern rainforests to the western dry forests. They hibernate either in tree hollows or buried underground between 3.5 and 8 months, depending on the seasonality of their habitats (Blanco et al., 2013; Dausmann, 2013; Dausmann and Blanco, 2016). As applies to most hibernators, this strategy requires preparation as well as sufficiently favorable conditions for recovery when hibernation is terminated, and reproduction needs to commence promptly. Thus, it can only be expressed in fairly predictable habitats. More flexible responses are shown by the mouse lemurs (*Microcebus*- over 20 species at present count). Most likely, all species are heterotherms and every mouse lemur species studied up to date has shown some form of seasonal torpor (**Table 1**). Some of these species have also shown months-long hibernation, however, in each case, only some individuals in a population did so, and individuals alternated between occasional torpor, short bouts of torpor, prolonged torpor and months-long hibernation both within and between winter seasons (Schmid and Ganzhorn, 2009; Kobbe et al., 2011). *Allocebus* and *Mirza* species have been reported or presumed to exhibit daily torpor during winter, albeit at different and flexible frequencies, however data have yet to be recorded (Dausmann and Warnecke, 2016; **Table 1**).

Most lower latitude hibernators, such as many bat species (see **Table 1**), short-beaked echidnas, *Tachyglossus aculeatus* (Grigg et al., 1989), and eastern pygmy-possums, *Cercartetus nanus* (Turner et al., 2012), are not strictly seasonal in their use of torpor and though they will use hibernation in winter, they also undergo shorter bouts of torpor during the rest of the year (see Opportunistic Torpor below). However, like the lemurs mentioned above, there are some species, which show a strict seasonal use of torpor (either daily or prolonged torpor) such as the Southern African hedgehog, *Atelerix frontalis* (Hallam and Mzilikazi, 2011), the African lesser bushbaby, *Galago moholi* (Nowack et al., 2010, 2013b), pygmy slow loris, *Nycticebus pygmaeus* (Streicher, 2004; Ruf et al., 2015), the Damaraland mole-rat, *Fukomys damarensis* (Streicher, 2010), and the Mountain pygmy possum, *Burramys parvus* (Geiser and Broome, 1991). However, it has to be noted that sufficient data on year round torpor use are lacking for most species with most measurements restricted to the winter when torpor is expected (Levesque et al., 2016).

OPPORTUNISTIC TORPOR

Similar to the tenrecs and echidnas mentioned above, a number seasonal heterotherms also employ torpor independent of season, if the conditions are sufficiently challenging (**Table 1**). Opportunistic torpor enables these species to respond promptly to unpredictable environmental changes, such as prolonged droughts or cold spells, as well as to an unusual shortage of food. For example, the eastern and rock elephant shrews, *Elephantulus myurus* and *E. rupestris*, routinely show short daily bouts of torpor during the winter, but also use daily torpor opportunistically throughout the year, presumably triggered by low temperatures and high air humidity (Mzilikazi and Lovegrove, 2004; Oelkrug et al., 2012). Furthermore, echidnas and marsupial antechinus (*Antechinus* spp.) have recently been reported to intensify torpor use in response to the threats of and the reduced food availability after fires (Stawski et al., 2015; Nowack et al., 2016). Other species, such as sugar gliders, *Petaurus breviceps,* or golden spiny mice, *Acomys russatus,* have been observed to use or intensify torpor in response to storms or floods (Nowack et al., 2015; Barak et al., 2019). Similarly, many non-Holarctic bat species exhibit prolonged torpor during winter (up to several days; reviewed by Geiser and Stawski, 2011) while also using opportunistic short bouts of torpor in other seasons. For example, the Northern long eared bat, *Nyctophilus bifax*, uses prolonged torpor (up to 5.4 days) during winter, but short bouts of torpor in response to cold weather conditions during summer (Stawski et al., 2009). Another, only recently studied, example is the Malagasy bat, *Macronycteris commersoni*. This species roosts in hot caves (≥32°C year-round) and displays a whole spectrum of different torpor responses during summer and winter (Reher et al., 2018). In summer, individuals may remain euthermic or enter torpor bouts lasting up to 6 days (sometimes coinciding with cyclones), while in winter their responses range from short torpor bouts, over prolonged torpor to hibernation with single bouts lasting up to 16 days; the triggers for the duration of torpor use remain unknown (Reher et al., unpublished data).

Other species also show opportunistic torpor independent of season, but in response to ambient conditions or food and water supply (**Table 1**). Amongst those are the pouched mouse, *Saccostomus campestris,* which entered torpor in the laboratory over a wide range of ambient temperatures and independent on photoperiod (Lovegrove and Raman, 1998; Mzilikazi and Lovegrove, 2002), the pichi, *Zaedyus pichiy,* which has been reported to use short bouts of torpor in spring after the hibernation season (Superina and Boily, 2007), the monito del monte, *Dromiciops gliroides* that entered torpor in the laboratory when kept under long photoperiod and relatively warm temperatures (20°C) (Bozinovic et al., 2004; Nespolo et al., 2010), and the striped faced dunnart, *Sminthopsis macroura* (Geiser and Baudinette, 1987; Song et al., 1997). In contrast, opportunistic use of heterothermy has been observed in only a handful of Holarctic species. The edible dormouse, *Glis glis*, for example, has been shown to re-enter hibernation already in July and to remain torpid until the following year (up to 11 months in total) in non-mast years when reproduction is skipped (Bieber and Ruf, 2009; Hoelzl et al., 2015).

RARE USE OF TORPOR

Not all heterotherms use torpor on a regular basis. A few species are known to be physiologically capable of employing torpor but do so only under adverse conditions and instead remain homeothermic whenever possible. A well-studied example is the African lesser bushbaby, *Galago moholi*. This species was long thought to be strictly homeothermic, despite cool environmental temperatures and a lack of food present in its habitat during winter. However, Nowack et al. (2010) detected sporadic daily torpor in a small fraction of the population. Since even these few (primarily juvenile and subadult) individuals became torpid only on single occasions, this suggests an unusual or specific trigger and is clearly not a regular seasonal response (Nowack et al., 2010, 2013b). Instead, behavioral and nutritional strategies are used by *G. moholi* to facilitate survival in winter, including larger sleeping groups, better insulated sleeping places, and changes in diet (Nowack et al., 2013c). In *G. moholi*, heterothermic phases are always shorter than 24 h and individuals have unusual difficulties rewarming to euthermic levels on cold days. The fact that *G. moholi* possess brown adipose tissue and the ability to use non-shivering thermogenesis suggests that individuals only enter torpor when their internal energy stores are depleted and that they have to rely on exogenous heat to return to active T_b (Nowack et al., 2013a).

Other heterothermic species which have been found to use torpor only rarely are the sugar glider, *Petaurus breviceps* (Christian and Geiser, 2007) and the feathertail glider, *Acrobates pygmaeus* (Jones and Geiser, 1992). Torpor use in *P. breviceps* seems to be triggered by especially cold and rainy days when animals remain in their nests instead of foraging (Körtner and Geiser, 2000; Nowack et al., 2015), enabling them to occur over a wide range of climatic conditions and even in cold temperate areas (snow and freezing conditions overnight). *A. pygmaeus* commonly exhibit bouts of short torpor but seem to be able to extend torpor use and undergo prolonged bouts when cold stressed (8–12°C; Jones and Geiser, 1992). Further examples of reluctant use of torpor are found in the rodent family. Although most Australian ash-gray mice, *Pseudomys albocinereus*, remained euthermic over a range of ambient temperatures when studied in the laboratory, one individual became torpid at 20 and 25°C (Barker et al., 2012). Body temperature recordings also suggest that Australian bush rats (*Rattus fuscipes*), which have a large geographical distribution, including tropical moist forests, can use torpor to compensate for lost feeding opportunities (unpublished data Nowack and Turbill). On the other hand, sandy inland mice (*Pseudomys hermannsburgensis*), who are also endemic to Australia, responded to food deprivation and low ambient temperature by becoming hypothermic without being able to arouse spontaneously (Tomlinson et al., 2007). More in-depth studies of species with large latitudinal ranges will provide better understanding of the flexibility of these responses.

Heterothermy as a singular response to acute emergency situations has to our knowledge not been reported for Holarctic mammals. This strategy, however, could be conceivable for members of the Sciuridae, which includes the classic hibernators (ground squirrels and chipmunks in the clade Marmotini). Interestingly, tree squirrels (sub-family Sciurinae) including European red squirrels (*Sciurus vulgaris*), have not been observed to enter torpid states (Dausmann et al., 2013), although it appears likely that closely-related flying squirrels are capable of shallow torpor (Olson et al., 2017). Tropical and sub-tropical sciurids have not been studied and more field studies are clearly needed to elucidate the potential for rare use of torpor in otherwise homeothermic mammals.

Differences in Body Temperature Are Related to Climate

One could assume that the clearest difference between tropical and Holarctic heterotherms is that T_b does not fall to extremely low levels. Indeed, with the exception of higher elevation habitats, ambient temperature rarely falls to life-threating temperatures below 0°C in the tropics even during winter, and thus individuals should not need mechanisms to avoid freezing. The record holders, in terms of low T_b, amongst mammals are the Arctic ground squirrel, *Spermophilus parryii*, and the European hazel dormice, *Muscardinus avellanarius*, both regulating a decrease in T_b down to as low as −2.9°C during hibernation without freezing (Barnes, 1989; Pretzlaff and Dausmann, 2012). Nonetheless, heterotherms from non-temperate or arctic areas can sometimes approach this temperature, and the southern African hedgehog, *Atelerix frontalis*, which is also one of the largest of all subtropical heterotherms (400–800 g), has been observed to hibernate with a T_b as low as 1°C (Hallam and Mzilikazi, 2011). In lemurs and tenrecs, the lowest T_b during hibernation recorded to date is 6.5°C in *M. griseorufus* (**Table 1**; Kobbe et al., 2011), but more commonly T_b is higher between 10°C and 30°C. Indeed, lemurs and tenrecs continue to hibernate even at T_bs >30°C (**Figure 2**). Similarly, eastern pygmy-possums (*Cercartetus nanus*) become torpid within their thermo-neutral zone and golden spiny mice (*Acomys russatus*) even use torpor at ambient temperatures as high as 35°C (Song et al., 1997; Grimpo et al., 2013).

To imperically test for differences in minimal torpid T_b and torpid metabolic rates between Holarctic and non-Holarctic species we used the "plgs" function in the R package "caper"[R version 3.6.2 (Orme et al., 2013; R Development Core Team, 2019)] on data from species in **Table 1** and Holarctic species from Ruf and Geiser (2015) and controlled for phylogenetic relatedness using the mammal phylogeny by Upham et al. (2019). We tested for differences between torpor T_b using "tbmin∼distribution" and in metabolic rate using "torpidMR∼mass+distribution." Holarctic species had significantly lower minimum torpid T_b ($p = 0.001$), and torpid metabolic rates ($p < 0.0001$) than non-Holarctic species, despite a high degree of overlap in both values between the two groups (**Figure 3**). However, in most, if not all, of these examples from non-Holarctic species the lowest T_b measured was bounded at the lower end by the coldest ambient temperatures, it is therefore difficult to predict if torpor patterns in non-Holarctic species would be similar at colder temperatures. The study of tropical heterotherms emphasizes that heterothermy must be defined on the basis of mechanistic, physiological parameters, such as significantly decreased metabolic rate (or heart rate), and not solely by T_b (Dausmann et al., 2004; Kobbe et al., 2011; McKechnie and Mzilikazi, 2011; Canale et al., 2012). However, our analysis has shown that we only have metabolic rate data from about half of the studied species (52% Holarctic and 57% of Non-Holarctic). Furthermore, the metabolic rate data in **Table 1** do not necessarily represent minimum torpid metabolic rate for a species as they might have been taken in one study while longer/deeper torpor bouts have been recorded in another study in which only T_b has been measured (as in the case of *Acomys russatus*) or because metabolic rate data have been obtained under a (field-)laboratory setup while T_b has been obtained from free-ranging animals; in fact only eight studies that report torpor metabolic rates of non-Holoractic species have been measuring metabolic rates of animals in the field.

The low risk of freezing in most tropical heterotherms has consequences for several aspects of their physiology including fat metabolism. In the fat-tailed dwarf lemur, *C. medius*, the main fuel during hibernation is monounsaturated oleic acid, which is synthesized preferentially from dietary carbohydrates obtained before food supply diminishes (sugary fruits; Fietz et al., 2003). This fat is stored in large quantities in the tails as triglycerides, resulting in an almost doubling of body mass (Fietz and Dausmann, 2007). Temperate hibernators, on the other hand, profit from a high content of polyunsaturated fatty acids (PUFAs) during hibernation. A diet rich in n-6 PUFAs has been shown to lead to longer and deeper torpor bouts (Geiser and Kenagy, 1987; Giroud et al., 2013) and thus higher energy savings during winter as they increase the activity of the sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) and thus counteract the suppressed enzymatic

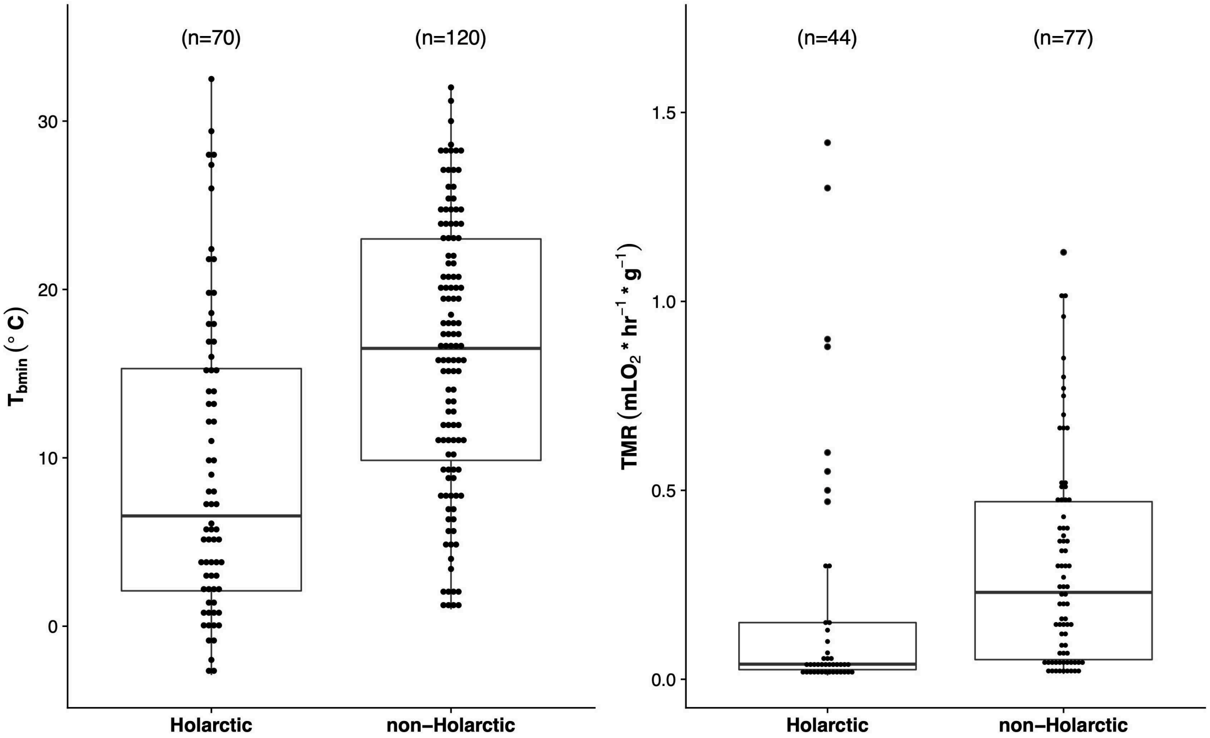

FIGURE 3 | Boxplots (representing the minimum, maximum, median, first quartile and third quartile) of minimal torpid body temperatures (T_b;°C) and minimal torpid metabolic rate $T_b$min for the non-Holarctic species with additional Holarctic species described by Ruf and Geiser (2015). Values for both were, on average, significantly higher in non-Holarctic species (see text).

activity and allow for greater fluidity of cell membranes at low temperature. Interestingly, n-3 PUFAs have a so far unexplained negative effect on torpor use (Giroud et al., 2013, 2018) and a diet rich in n-3 PUFAs has been shown to not only significantly reduce torpor use in temperate zone heterotherms but also in the tropical daily heterotherm, *Microcebus murinus* (Vuarin et al., 2016). However, the amount of n-6 and n-3 PUFAs in the diet is unlikely to be critical for tropical heterotherms as they usually do not hibernate at T_bs close to freezing (Goldman, 1975; Frank, 1991; Vuarin et al., 2016). They may thus avoid autoxidation and the production of toxic fatty acids peroxides, which are by-products of the metabolism of essential fatty acids.

Different Triggers of Torpor Use

Although torpor use in temperate and arctic regions is usually triggered by photoperiod, low temperatures, and limited food ability, the data summarized in **Table 1** clearly shows the variety of factors that are triggering torpor in non-Holarctic species. As mentioned above, even during winter months, cold does not seem to be a necessity for tropical and subtropical hibernators and torpor bouts are often caused by seasonal low water availability instead of low food availability. Interestingly, mouse-tailed bats (*Rhinopoma*) are even considered incapable of entering torpor during cold periods (Kulzer, 1965). Levin et al. (2015) found that two species of mouse-tailed bats (*Rhinopoma cystops*, *Rhinopoma microphyllum*) hibernated in a geothermally heated cave in winter with a stable ambient temperature of 19–23.8°C although colder caves were available. Both species showed the lowest torpor metabolic rate at ~20°C and aroused at ambient temperature below 16°C (Levin et al., 2015). Thus, the availability of warm caves has allowed them to expand their subtropical distribution range from semi-arid and warm regions in Asia and Africa into southern Israel, the northern edge of their distribution (Levin et al., 2008, 2015). Similarly, the lesser long-eared bat (*Nyctophilus geoffroyi*) uses short bouts of torpor in tropical northern Australia in winter where ambient temperatures do not drop below 16.5°C (Geiser et al., 2011). Opportunistic torpor is often triggered by unpredictability in environmental parameters, such as unseasonal food or water shortages (e.g., droughts or fires), conditions that are less frequently encountered on an unpredictable basis in temperate and arctic regions.

The Advantages of Passive Arousals

Torpor at relatively high ambient temperatures still offers energetic savings, but without some of the major disadvantages. Many vital bodily functions can continue to operate at the comparatively high torpid T_b of non-Holarctic heterotherms and warming to euthermic levels is relatively inexpensive because animals can mostly rely on energy-saving, passive rewarming (Dausmann et al., 2009). An Australian desert bat, the inland free-tailed bat *Ozimops petersi* (former Mormopterus species 3; Lumsden, 2019), for example, can arouse from torpor without an obvious active thermogenic support (Bondarenco et al., 2013). Moreover, individuals can start being active at relatively low T_b, and then activity itself can be used as a means of heat production. Ortmann et al. (1997) first observed the so-called two-step arousal in a species of mouse lemur (*M. murinus*). Individuals rewarming from torpor initially use passive means via exogenous heat sources to raise their T_b to about 27°C before employing endogenous heat production, which keeps metabolic rate (and energy expenditure) during the rewarming process low. Since then, this mechanism has been verified in many other tropical heterotherms that closely synchronize their arousal times with the onset of rising environmental temperature which they may supplement by basking (Lovegrove et al., 1999; Schmid, 2000; Geiser et al., 2002; Mzilikazi et al., 2002; Geiser and Drury, 2003; Dausmann et al., 2005; Lovegrove and Génin, 2008; Warnecke et al., 2008; Kobbe et al., 2011; McKechnie and Mzilikazi, 2011; Nowack et al., 2013b; Dausmann et al., submitted). Although mostly observed in species inhabiting relatively warm habitats, a recent study found that Djungarian hamsters (*Phodopus sungorus*), originally inhabiting Asian steppes, actively seek the heat of a basking lamp when rewarming from torpor under laboratory conditions (Geiser et al., 2016). This finding suggests that the use of passive rewarming might also be common in Holarctic heterotherms.

Perhaps one of the most surprising discoveries from warm climate hibernators is that periodic arousals, thought to be essential to proper functioning during hibernation, are not necessarily needed (Dausmann et al., 2005; Lovegrove et al., 2014b). Depending on the nature of the hibernaculum (insulative capacities) and ambient temperature, the T_b of tropical species can fluctuate widely during hibernation (daily fluctuations of T_b can exceed 25°C per day). Under these conditions, when T_b occasionally rises passively above about 30°C, the expensive arousals are abandoned in *Cheirogaleus* (**Figure 2**). In contrast, individuals hibernating in better insulated sites (large trees or underground), which have a lower but more stable T_b, show regular periodic arousals, just like their temperate or arctic counterparts (Dausmann et al., 2004, 2005). Similarly, the common tenrec (*Tenrec ecaudatus*) forgoes arousal completely while hibernating in an underground burrow where T_bs remain above 25°C for the winter (Lovegrove et al., 2014b). Although the ultimate factors necessitating periodic arousals in hibernators remain enigmatic (proposed explanations include reduction of oxidative stress and sleep debt, production of gene products, activation of the immune system, limitation of neurophysiological damage; Carey et al., 2003), it seems clear that the capacity to attain a high T_b passively determines whether or not arousals are required (Dausmann, 2014). For example, arctic ground squirrels, *S. parryii*, consistently sleep during arousals (Daan et al., 1991). Their electroencephalography shows the decrease in slow wave activity as would be expected when sleep debt had increased during the preceding phase of torpor. Thus, it has been postulated that the need for sleep slowly accumulates during torpor and that returning to euthermy is periodically required to sleep (Daan et al., 1991; Trachsel et al., 1991). Indeed, *C. medius* display aspects of sleep (rapid eye movement sleep) during the phases of passively heated warmer T_b during hibernation (in contrast to phases of low T_b), possibly preventing the accumulation of sleep debt, and ensuring brain function and memory consolidation in individuals with highly fluctuating T_b (Krystal et al., 2013). To date, the only other mammal to not exhibit periodic arousals besides hibernating lemurs and tenrecs (Dausmann et al., 2004; Lovegrove et al., 2014b) are black bears, *Ursus americanus*, hibernating at T_b of >30°C (Tøien et al., 2011). The opportunity to abandon periodic arousals not only saves energy, but also limits the damaging high level of oxidative stress resulting from the increased production of reactive oxygen species during active rewarming (Carey et al., 2003; Giroud et al., 2009).

INTER- AND INTRASPECIFIC PHENOTYPIC FLEXIBILITY OF TORPOR USE IN THE TROPICS

Most interestingly, the distinction between the use of short torpor bouts and hibernation is less clear-cut in non-Holarctic heterotherms (Mzilikazi and Lovegrove, 2004; Cory Toussaint et al., 2010; Kobbe et al., 2011; Canale et al., 2012). Whereas, most Holarctic species usually either hibernate or use daily torpor, warmer climate species often show a mixed use of shorter and longer bouts. In the tropics, there is more variation between closely related species at the same site, between populations of the same species at different sites, between individuals within a population, and even in individuals between years (Dausmann, 2014). As outlined above, some species can switch between hibernation (several months), prolonged torpor over several days, becoming torpid for a few hours sporadically or daily (opportunistic) use of short torpor bouts, or remaining homeothermic. As the best examples of this phenotypic flexibility come from southern Madagascar, the driest and least predictable habitat of the island, we suggest that this flexibility may enable tropical species to inhabit not only seasonal, but also unpredictable habitats. It enables species to respond to the context of specific environmental parameters and their own body condition. For example, in the lemur *Microcebus griseorufus* and the bat *Macronycteris commersoni* all of these responses have been observed in different individuals in the same population at the same site (Kobbe and Dausmann, 2009; Kobbe et al., 2011; Reher et al., 2018). Similarly, western rock elephant shrews, *E. rupestris*, are highly heterothermic in South Africa, whereas the closely-related cape rock elephant shrews, *E. edwardii*, at the same site remain mostly homeothermic, although the species is capable of short to prolonged bouts of torpor (McKechnie and Mzilikazi, 2011; Boyles et al., 2012). Hottentot golden moles, *Amblysomus hottentotus longiceps*, and southern African hedgehogs, *A. frontalis*, seem to be capable of diverse thermoregulatory responses at the same site, and under the same conditions in captivity (Scantlebury et al., 2008; Hallam and Mzilikazi, 2011). Despite hibernation being a more fixed response, there is also variation in thermoregulatory patterns during hibernation, such as in dwarf lemurs and tenrecs, where the insulative properties of the hibernacula determine the occurrence and extent of daily T_b fluctuations and the occurrence of periodic arousals.

Furthermore, the duration of hibernation can be flexible between different populations of the same species, depending on the duration of the period of scarceness in the particular habitat. For example, *C. medius* hibernates up to 8 months in the western dry forests of Madagascar, but only 3.5 months in the south-western littoral forests, where the dry season is much shorter (Lahann and Dausmann, 2011). Differences in thermoregulatory patterns might also emerge as a result of competition with other, closely related sympatric species. Only half as many individual *M. griseorufus* entered hibernation in a habitat where the larger *M. murinus* is present in the littoral forest in the south of Madagascar, compared to where *M. griseorufus* occurs alone the spiny forest of the south-west (Kobbe et al., unpublished data). As hibernation is thought to be the more advantageous strategy in *Microcebus* species (higher energy savings; predation avoidance), *M. griseorufus* could be outcompeted for access to food before the hibernation period by the larger *M. murinus*, limiting pre-hibernation fattening. If true, then *M. griseorufus* may have to resort to short or prolonged torpor episodes to cope with the Malagasy winter in areas where it occurs together with *M. murinus*.

TROPICAL ORIGINS OF HETEROTHERMY, AND ENDOTHERMY, IN MAMMALS

It now seems increasingly likely that endothermy in mammals evolved from the ancestral ectothermic condition via a heterothermic state (Grigg et al., 2004; Geiser and Stawski, 2011; Lovegrove, 2017). All extant mammals are capable of metabolic heat production (Lovegrove, 2012), but the degree to which they regulate their T_b, both in terms of absolute temperature and level of variability, varies considerably (Boyles et al., 2013; Clarke and O'Connor, 2014). Mammals evolved under climatic conditions similar to modern day tropics, where ambient temperature is high for most of the year, and the costs of maintaining a comparatively high, stable T_b, especially at a lower level (~32°C like in tenrecs and echidnas) would be relatively low (Levesque et al., 2013). This notion is supported by the fact that early mammalian ancestors had small body masses, were nocturnal, and mostly insectivorous (McNab, 1978; Kemp, 2006; O'Leary et al., 2013), similar to the hedgehog tenrecs. Moreover, it has been postulated that small mammals in particular cannot sustain the high rates of evaporation necessary to maintain a T_b more than 2°C below ambient for more than a few hours (Crompton et al., 1978), because of substantial water loss needed for evaporative cooling. Often, tropical and subtropical heterotherms inhabit dry environments and water savings might even be more essential for survival than energy savings (Schmid and Speakman, 2000; Dausmann, 2014). Water loss, however, could be avoided if T_b is regulated slightly above ambient, and the outward flow of heat is varied by insulation, therefore, a switch to a diurnal activity pattern in some species would have necessitated higher T_bs (Crompton et al., 1978), which in turn were only made possible with the evolution of the scrotum (Lovegrove, 2019). The evolution of endothermy in mammals is an emergent property of the evolution of various characteristics that aid in either heat production (e.g., thermogenesis, UCP1) and heat dissipation (e.g., insulation, external scrotums) and happened to a different degree across the mammalian lineage (Lovegrove, 2012, 2019; Seebacher, 2018; Jastroch and Seebacher, 2020). By studying the added level of variability in many thermoregulatory traits, torpor use in particular, observed in species in the tropics and sub-tropics can help shed further light on how endothermy evolved in mammals.

Although advances in understanding the ecological and physiological underpinnings of tropical heterothermy have been substantial, many questions remain. For example, we need to learn more about how blood parameters (lactate levels, as a proxy for hypoxia in the tissue), immunocompetence and sleeping patterns in tropical hibernators differ compared to hibernators with continuously low T_b, especially if we wish to disentangle hibernation specific and low T_b specific physiological responses, which could help elucidating the evolution of homeothermy.

AUTHOR CONTRIBUTIONS

JN and KD conceived of the idea and wrote the first version of the manuscript. DL ran the analyses and prepared the figures for the manuscript. All authors contributed to the final version of the manuscript.

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REVIEW
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Physiological, Behavioral, and Life-History Adaptations to Environmental Fluctuations in the Edible Dormouse

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The edible dormouse (*Glis glis*, formerly *Myoxus glis*) is a small arboreal mammal inhabiting deciduous forests in Europe. This rodent shows behavioral and physiological adaptations to three types of environmental fluctuations: (i) predictable seasonal variation in climate and food resources (ii) unpredictable year-to-year fluctuation in seed-production by trees and (iii) day-to-day variation in ambient temperature and precipitation. They cope with seasonally fluctuating conditions by seasonal fattening and hibernation. Dormice have adjusted to tree-mast fluctuations, i.e., pulsed resources, by sensing future seed availability in spring, and restricting reproduction to years with at least some seed production by beech and oak trees, which are a crucial food-resource for fast-growing juveniles in fall. Finally, dormice respond to short-term drops in ambient temperature by increased use of daily torpor as well as by huddling in groups of up to 24 conspecifics. These responses to environmental fluctuations strongly interact with each other: Dormice are much more prone to using daily torpor and huddling in non-reproductive years, because active gonads can counteract torpor and energy requirements for reproduction may prevent the sharing of food resources associated with huddling. Accordingly, foraging activity in fall is much more intense in reproductive mast years. Also, depending on their energy reserves, dormice may retreat to underground burrows in the summers of non-reproductive years, causing an extension of the hibernation season to up to 11.4 months. In addition to these interactions, responses to environmental fluctuations are modulated by the progression of life-history stages. With increasing age and diminishing chances of future reproduction, females reproduce with increasing frequency even under suboptimal environmental conditions. Simultaneously, older dormice shorten the hibernation season and phase-advance the emergence from hibernation in spring, apparently to occupy good breeding territories early, despite increased predation risk above ground. All of the above adaptions, i.e., huddling, torpor, hibernation, and reproduction skipping do not merely optimize energy-budgets but also help to balance individual predation risk against reproductive success, which adds another layer of complexity to the ability to make flexible adjustments in this species.

Keywords: pulsed resources, tree masting, seasonal adaptation, hibernation, torpor, huddling, reproduction skipping

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INTRODUCTION

Constant environments are rare. Consequently, animals commonly have to cope with environmental fluctuations. These fluctuations are predictable if they originate from celestial motions of the earth, such as yearly and daily cycles in solar radiation. In many cases, however, variation of the environment, such as fluctuations in local food availability or short-term weather conditions, can be largely unpredictable. Natural selection has therefore favored rheostasis, i.e., the ability of animals to respond to environmental fluctuations by repeatedly changing and readjusting regulated physiological and behavioral levels (Mrosovsky, 1990). These adjustments can affect both energy intake, e.g., foraging behavior or digestive function, and energy expenditure, e.g., the regulation of metabolic rate, body temperature or locomotor activity.

Among mammals, challenging environmental conditions will have a particularly strong impact on small species. This is because of high rates of heat loss, due to a high body surface to volume ratio, which render small endotherms highly susceptible to cold load. At the same time, their high energy demands mean that small mammals can quickly suffer from shortages of food (e.g., Bronson, 1987). A prime example of small mammal exposed to multiple environmental fluctuations is the edible dormouse, a ∼100 g mammal with an arboreal lifestyle that inhabits deciduous forests in western and central Europe. *Glis glis* is an ancient mammal and belongs to the *Gliridae*, which diverged from the *Sciuroidea* at the transition between Paleocene and Eocene, ∼55 MYA, at the time of general rodent diversification (Huchon et al., 2002). The rise of and diversification of the *Gliridae* was likely enhanced by the warm climate in Eurasia that supported the spread of glirid-favored deciduous forests (Nunome et al., 2007). Subsequently, overall earth surface temperatures have considerably declined (Zachos et al., 2001). Simultaneously, edible dormice have acquired a number of rheostatic responses to flexibly adjust their energy turnover in response to environmental challenges. These responses include adaptive modifications in physiology, morphology, and behavior. Below, we examine major responses of this species, namely adaptations to regular seasonal environmental cycles, to unpredictable pulsed food resources, and to rapid fluctuations in weather conditions.

SEASONAL ADAPTATION

"Stuffed dormouse. Is stuffed with a forcemeat of pork and small pieces of dormouse meat trimmings, all pounded with pepper, nuts, laser, broth. Put the dormouse thus stuffed in an earthen casserole, roast it in the oven, or boil it in the stock pot" (Vehling, 2012). This recipe in the collection "*De re coquinaria* (On the Subject of Cooking)," which is thought to have been compiled in the first century AD, nicely illustrates the origin of the common name "edible dormouse" for *G. glis*. Dormice were probably considered a delicacy in the Imperial Rome because this hibernator can accumulate particularly large amounts of fat. The ancient Romans fattened dormice in earthen jars (*Dolia*) and seemed even to compete for the heaviest dormouse at banquets (Carpaneto and Cristaldi, 1995). For instance, in central Europe (Austria), free-living adult dormice emerge in spring (April/May) with a body mass of 91.1 ± 1.2 g, and reach 128.1 ± 1.1 g prior to hibernation (September/October; Ruf and Bieber, unpublished, n = 953, data from 13 years). Some individuals reach a body mass of >200 g prior to hibernation in the field (Löhrl, 1963, Ruf and Bieber, unpublished) as well as in captivity (Bieber and Ruf, 2009b). On average, there is a 40% gain of body mass over the summer season, which appears to be entirely due to the deposition of white adipose tissue (Schaefer et al., 1976). This puts body fat reserves in dormice in the same range (30–50%) as in phocid seals at parturition, which are well known to use extremely large maternal lipid reserves for lactation at sites far removed from food resources (Oftedal, 2000).

Given the allometry of energy expenditure, a small mammal storing large amounts of body reserves is well adapted only if their use is associated with a profound reduction of metabolic rate. Consequently dormice, like many other small mammals, enter states of torpor during hibernation in which energy expenditure is temporarily lowered to only ∼5% of basal metabolic rate (Ruf and Geiser, 2015). Immediate energy savings during the torpid state will be even higher because they allow the animals to avoid maintaining large body-to-ambient temperature gradients during winter. During hibernation, dormice abandon their nocturnal, arboreal activity, cease foraging, and retreat to underground caves or self-dug hibernacula, mostly solitary but occasionally communally in groups of 2–3 (Jurczyszyn, 2007; Kryštufek, 2010; Trout et al., 2018). Thus, hibernation is the most conspicuous behavioral response of dormice to seasonal cold and shortness of food.

Hibernation duration in *G. glis* is relatively long and variable (7–9 months) (Vietinghoff-Riesch, 1960; Kryštufek, 2010; Bieber et al., 2018). Part of this variability is due to the occurrence of reproduction, which delays the onset of hibernation among adults of both sexes (Bieber et al., 2018). Variation in hibernation patterns is also caused by differences in ambient temperature (T_a). First, the duration of torpor bouts that are periodically interrupted by arousals strongly depends on T_a, in *G. glis* as well as in other hibernators (French, 1982; Buck and Barnes, 2000; Bieber and Ruf, 2009b). As the frequency of arousals increases with T_a, this accelerates the depletion of energy stores, since warming up from torpor is costly (Wang, 1979; Strijkstra, 1999; Nowack et al., 2019). Ultimately, this energetic impact of warm temperatures, which does affect the animals in relatively shallow (∼30–80 cm) burrows (Jurczyszyn, 2007; Bieber and Ruf, 2009b; Hoelzl et al., 2015) may force the animals to terminate hibernation early. Second, the seasonal cycle of hibernation as well as of body mass or gonadal function may involve an endogenous rhythm, although this rhythm under experimental conditions had a period of ∼6 months only (Scott and Fisher, 1976; Jallageas et al., 1989). The synchronizing zeitgeber for this rhythm apparently is not photoperiod, however, like in most other hibernators, but seems to be the yearly cycle in T_a, which is why edible dormice have been called thermoperiodic (Jallageas et al., 1989). Both effects of T_a may cause flexible adjustment of the pattern and duration of hibernation to climatic conditions. Presently, however, it remains to be seen whether the duration of the hibernation season differs as systematically with geographical distribution and climate as seems to be the case in the closely related garden dormouse (Giroud et al., 2014).

A third, and quite different, cause of variation in the use, pattern, and duration of hibernation are trade-offs linked to the use of torpor. Drawbacks of torpor are reflected by mammals avoiding or minimizing this strategy if external or body energy reserves permit (Humphries et al., 2003; Bieber et al., 2014; Zervanos et al., 2014). Dormice with high body mass, for instance, minimize torpor bout duration and regulate higher minimum T_b during hibernation. Negative consequences of torpor may include sleep deprivation, impaired immune function and oxidative stress (e.g., Daan et al., 1991; Deboer and Tobler, 1994; Carey et al., 2000; Prendergast et al., 2002; Blagojevic et al., 2011). In dormice, oxidative stress seems to be particularly associated with rewarming from deep T_b which leads to the shortening of telomeres, the protective endcaps of chromosomes (Hoelzl et al., 2016a; Nowack et al., 2019). Telomeres can be elongated, but this is energetically costly, which creates a powerful trade-off between the costs and benefits of hibernation at low T_b (Hoelzl et al., 2016a; Nowack et al., 2019).

Energy savings are, however, not the only benefit of hibernation, this way of overwintering is also a predator-avoidance strategy. Free living edible dormice reach a maximum longevity of 14 years, which is long for a small rodent (Trout et al., 2015). Longevity is, however, quite typical for small hibernators (Turbill et al., 2011). It may seem peculiar that it should pay for small rodents to allocate resources to somatic maintenance to increase longevity, given that they typically suffer from high extrinsic mortality, namely via predation (Kirkwood, 1977). As it turns out, however, hibernators have much higher survival rates during winter than during the active season, and hence low overall extrinsic mortality (Turbill et al., 2011). Decreased winter-mortality is also typical for edible dormice, and this pattern occurs in different populations across Europe (Lebl et al., 2011a). Predator avoidance is presumably due to torpid animals remaining largely motion- and odorless in hibernacula, which makes them difficult to detect (Turbill et al., 2011; Ruf et al., 2012). This ability of deeply torpid mammals to evade predators has even been demonstrated experimentally (Brown, 1970).

To avoid predators, hibernators should remain hidden in hibernacula at times when predator activity is highest. In dormice, this is in early spring and early summer when their main predators, especially owls, are raising their young and exhibit peak foraging activity (e.g., Forsman et al., 1984). Mortality rates in edible dormice as well as in the closely related common dormice (*Muscardinus avellanarius*) are in fact highest at this time of the year (Lebl et al., 2011a; Bieber et al., 2012). This temporal pattern creates another trade-off since hibernators generally may benefit from early emergence in spring, because it allows individuals to occupy good territories and gain additional mating opportunities (Davis and Murie, 1985; Morbey and Ydenberg, 2001). Hence, early termination of hibernation can increase reproductive success in squirrels, marmots, and hamsters (Murie and Harris, 1982; Millesi et al., 1999; Franceschini-Zink and Millesi, 2008; Ozgul et al., 2010) and prolongs the time for pre-hibernation fattening.

The timing of the hibernation season, particularly the time of spring emergence should therefore result from conflicting needs for safety versus above-ground activity and reproduction. As it turns out, the outcome of this trade-off is age-dependent (**Figure 1**). With increasing age, edible dormice phase-advance the entire hibernation season, with a most pronounced forward-shift of spring emergence. Simultaneously, there is an increase of reproductive capability (functional testes) in males and the probability of having a litter (in females) with progressing age, both in outdoor enclosures and by free-living animals (Hoelzl et al., 2016b; Bieber et al., 2018). We attribute the increased likelihood of reproductive activity in older animals to increasing mortality and diminishing chances for future reproduction (Bieber et al., 2018), which are not only related to age *per se* but also to high year-to-year variation in food resources (see below). In other words, old dormice cannot afford to ‘sit tight’ until environmental conditions are optimal for reproduction. Thus, hibernation is not just altered by variation in energy stores or rates of energy expenditure, as outlined above. The interaction between age, reproduction and the timing of torpid states in dormice indicates that hibernation is also a life history trait, the use and timing of which is adjustable given an animals age and residual reproductive value.

The timing of emergence from hibernation in spring is also sex-specific (Vietinghoff-Riesch, 1960; Bieber, 1998; Schlund et al., 2002). In edible dormice, as in many hibernators, males emerge before females (Michener, 1983; Körtner and Geiser, 1998; Blumstein, 2009; Lane et al., 2011). Early emergence of males is thought to promote individual reproductive success by maximizing the number of potential mates available. Also, males have to spend several weeks at normothermic T_b after hibernation to complete spermatogenesis (Christian et al., 1972; Barnes et al., 1986).

The hibernation season is, however, merely one of several stages in a seasonal cycle in dormice. Other, equally important facets of this cycle include seasonal growth and shrinkage of alimentary organs such as the liver, which, along with the up- and downregulation of foraging activity contribute to the seasonal cycle of body energy reserves (Bieber et al., 2011, 2017). Also, dormice do not only reproduce seasonally but undergo gonadal involution during winter (Jallageas et al., 1992), which will further add to energy savings. Since all of these responses in mammals are interconnected, in fact they have been called an adaptive syndrome (Heldmaier and Lynch, 1986), it appears that any variation in seasonality is not only due to environmental factors but also affected by the state of animals within their life cycles.

PULSED RESOURCES

Edible dormice do not just feed on tree seeds, but also on foliage, flowers, pollen, fruits, berries, lichens, mushrooms, arthropods,

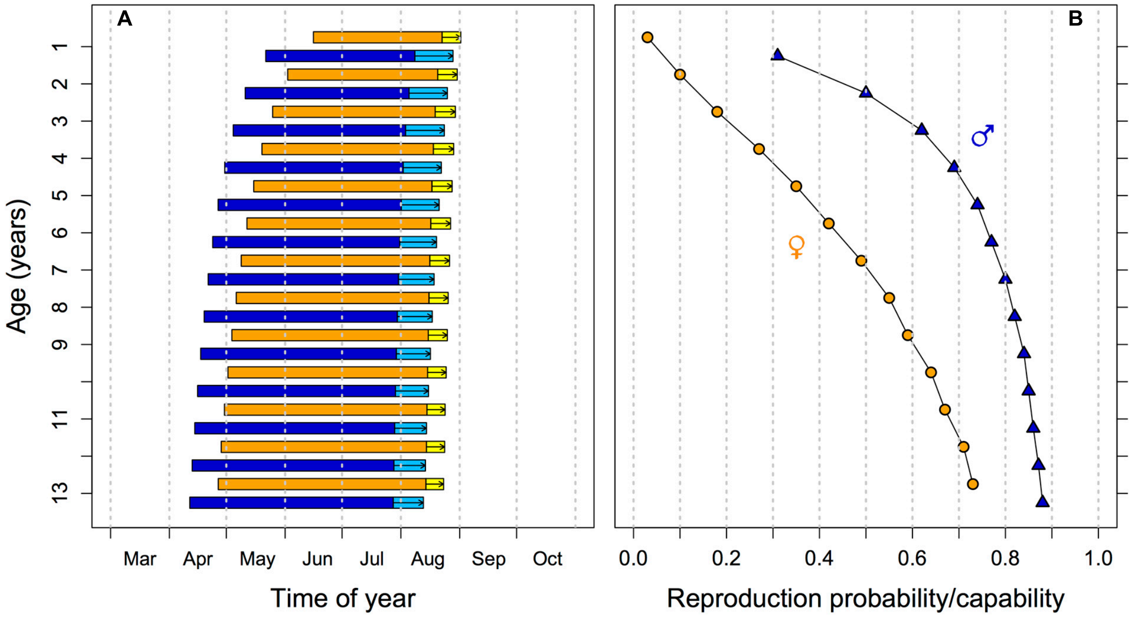

FIGURE 1 | Effects of age on **(A)** the timing of hibernation and activity and **(B)** on reproduction in yearlings and adult edible dormice. **(A)** As age increases both emergence from hibernation and hibernation onset occur earlier in the year, leading to a phase advancement of the active season (horizontal bars; blue: males; orange: females). Investment into reproduction leads to a delay in hibernation onset in both sexes (light blue and yellow bars with arrows). **(B)** The proportion of sexually active males with large testes (blue triangles) and of reproducing females (orange circles) increases with age. Predictions from multilevel models are based on data in Bieber et al. (2018).

and birds, which suffice to gain mass in non-reproductive years (Santini, 1978; Bieber, 1998; Koppmann-Rumpf et al., 2003; Fietz et al., 2005; Vekhnik, 2019). In fact, dormice populations can persist at sites with a very low seed-tree abundance, although with a lower lifetime reproductive output (Bieber and Ruf, 2009). Dormice do, however, respond strongly to year-to-year fluctuations in the mast seeding of deciduous trees, particularly beech and oak, by coupling reproduction to the availability of high-caloric seeds (Bieber, 1998; Schlund et al., 2002; Pilastro et al., 2003; Fietz et al., 2005; Ruf et al., 2006; Vekhnik, 2019). The fraction of successfully reproducing females is high in full mast years with virtually all trees seeding, average in intermediate mast years, when only a fraction of trees produce seeds, and entire populations of dormice can skip reproduction in mast failure years (see **Figure 2**; e.g., Fietz et al., 2005; Ruf et al., 2006).

The evolution of mast seeding of trees may have resulted from increasing wind pollination efficiency or from satiation of seed predators, such as mice, wild boar, or insects (Silvertown, 1980; Kelly et al., 2001). Regardless of the underlying reason, there are no easily detectable prior signs of masting in the subsequent year, which makes them an example of unpredictable pulsed resources (Ostfeld and Keesing, 2000). Despite this, edible dormice are adapted to these pulses, at least in beech forests, apparently by anticipating the availability of energy-rich seeds at the time of lactation in August through the consumption of unripe beechnuts just after emergence from hibernation in May (Bieber, 1998; Fietz et al., 2005). This explanation has been reinforced by a supplementary feeding experiment, in which the provision of high caloric seeds prior to parturition greatly increased the proportion of reproducing females and reproductively active males in an Austrian population (Lebl et al., 2010).

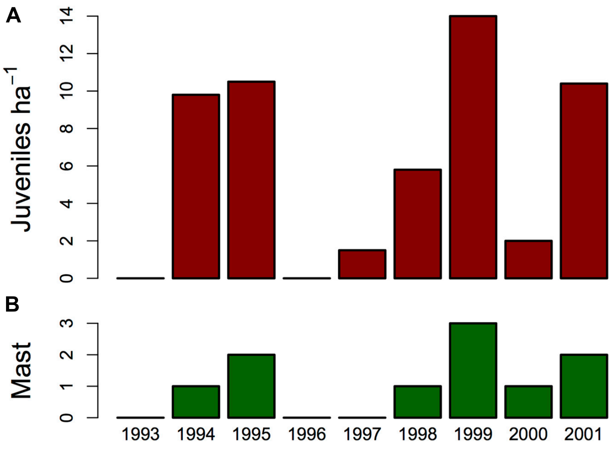

FIGURE 2 | (A) Year-to-year fluctuations in edible dormouse reproduction and density as determined by nest box controls over nine years at two study sites (data combined) in southwest Germany. Reproduction was low or even absent in 1993, 1996, 1997, and 2000. **(B)** This was correlated with a low or absent mast of beech trees. Bars represent the degree of beechnut production (codes 0–3 mean absence of beechnuts, some, good, and full mast, respectively). A rank correlation between this index and the number of juveniles detected per year was highly significant (Spearman's rho 0.965, *n* = 9, *P* = 0.007). Based on data in Ruf et al. (2006).

Thus, the presence of flowers or unripe seeds appears to be the signal that induces reproduction in dormice. Males respond to nutrient-rich food, even prior to the emergence of females, by the growth of testes (e.g., Bieber, 1998; Lebl et al., 2010). Whether the consumption of seed buds also triggers gonadal recrudescence in females is presently unclear, but in populations at the center of the distribution range there are no signs of sexual activity or pregnancy in females, irrespective of their body mass, in years of reproduction skipping (Bieber, 1998; Schlund et al., 2002). In this context, we tested the hypothesis that, in mast failure years, females may be forced to spend longer times foraging for low-quality food, which would increase their exposure to predators. Theoretically, this may lead to chronic stress, i.e., long-term increased secretion of glucocorticoids, which can have inhibitory effects on reproductive function in both female and male mammals (e.g., Boonstra, 2013). However, it turns out that females actually forage more intensively in full mast years (Bieber et al., 2017) and accordingly have higher stress-hormone levels in reproductive years (Cornils et al., 2018). Hence, stress hormones are not the proximate factor linking reproduction to beech seeding in dormice.

Certain aspects of the patterns observed in central/western European beech forests differ from the periphery of the distribution range, e.g., Russia. These peripheral populations also display extreme year-to-year fluctuations in reproduction, in this case linked to the seeding of oak (*Quercus robur*). However, in oak-dominated forests up to ~90% of males develop functional testes and most females gestate even in years of minimal reproductive output, but there is a synchronous mass resorption of embryos (Vekhnik, 2019). Vekhnik (2019) suggested that time constraints during the short active period in these habitats forces mating to occur rapidly after hibernation, when the degree of fruit or seed set of oak is uncertain, which is why the 'reproductive decision' must be made during gestation.

We suggest that the key to understanding different reproductive patterns of edible dormouse populations is in fact the degree of uncertainty about seed production. In beech, there is a clear correlation between flowering (as indicated by pollen abundance) and subsequent seed production. In contrast, in oaks, pollen abundance is not a predictor of acorn production. The amount of actual oak seed production is strongly affected by weather conditions (Kasprzyk et al., 2014; Bogdziewicz et al., 2017; Lebourgeois et al., 2018). We hypothesize that dormice in both beech and oak forests, actually under all environmental conditions, use the same simple tactic to regulate reproductive investments (**Figure 3**). In this scenario, individuals of both sexes will, upon emergence from hibernation, initiate investment into gonadal function and reproduction only if food resources with a high energy and nutrient content, e.g., inflorescences or seed buds, are abundant. Females will maintain this investment into reproduction only if high-quality food continues to be present, otherwise reproduction is aborted by embryo resorption. Due to the high reliability of beech, this tactic would lead to a population-wide reproduction versus reproduction skipping in beech masting and mast failure years, respectively. In oak-dominated forests it would lead to high rates of embryo resorption, whenever environmental conditions hamper the production and ripening of oak seeds, as observed in the Russian population (Vekhnik, 2019). Cases of embryo-resorption probably also occur in beech forests, particularly in intermediate mast years, but may well go unnoticed if they occur in early gestation. It would be highly interesting to investigate this question more closely by using imaging methods. Rather than absorbing embryos, occasionally, females may eat their pups after parturition if food is not available. This would explain observations of entire litters vanishing in an extremely hot summer in which beech nuts were present but empty, i.e., when even beech trees were an unreliable food source (unpublished). Of course, basing reproductive decisions on the continuous "sampling" of environmental resource availability may also be adaptive in other habitats inhabited by dormice, such as pine forests or stands with fruit-bearing trees (Santini, 1978; Bieber and Ruf, 2009a).

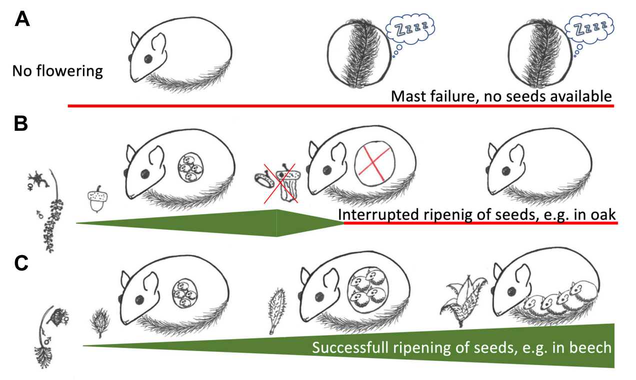

FIGURE 3 | Schematic depiction of reproductive decisions in edible dormice. **(A)** A situation where trees like beech or oak are not flowering, a mast failure year. In these years, reproduction will be skipped and aestivation and prolonged hibernation is observed. **(B)** In certain tree species, such as oak (*Quercus spec*.), there is no correlation between flowering and seeding. Under these circumstances, females may be gestating if seed buds are available but resorb embryos if the food availability decreases. **(C)** In beech (*Fagus sylvatica*) there is a strong correlation between flowering and actual seeding. Under these conditions, females experience a steadily increasing food availability, which allows high food availability for juveniles to fatten after birth.

In the beech forests of their central distribution the observed reproductive pattern (**Figure 2**) seems like an example of anticipatory reproduction, in which reproductive decisions are made before a resource pulse, i.e., ripe seeds, becomes available. An apparent prediction of future food abundance has been reported for a few other small mammals (e.g., Berger et al., 1981; Boutin et al., 2006; Bergeron et al., 2011). However, the existence of anticipatory reproduction has been disputed by White (2007, 2013), who pointed out that females require substantial amounts of protein to produce embryos, sustain their growth, and support the amount and quality of lactation. In the scenario outline above, this condition is met because the unripe seeds eaten by females, like all reproductive parts of plants, likely contain sufficient amino acid sources to initiate reproduction (Fietz et al., 2005; White, 2011). In addition to its role as a nutrient source, high-quality food may, however, also serve as an environmental signal that predicts the advent of ripe, energy-rich beechnuts in August–September, coincident with lactation. In this context, it would be interesting to assess whether dormice are particularly sensitive to the action of hormones that are known to serve as links between energy uptake, white adipose tissue and the reproductive system, such as leptin, ghrelin, and orexins (e.g., Kirsz et al., 2017; Williams et al., 2017).

Irrespective of whether or not the tactic employed by edible dormice may be called 'anticipatory reproduction,' there is no doubt that it serves to synchronize their annual peak in energy demand with the annual peak in resource availability, a central tenet in ecology (Thomas et al., 2001; Stenseth and Mysterud, 2002). In central Europe, edible dormice have only one litter per year, with a mean litter size of 5.5 young (Kager and Fietz, 2009; Lebl et al., 2010). Mating takes place from mid-June to mid-July; the gestation period lasts 30–32 days, and juvenile dormice are weaned at approximately 6 weeks of age (Koenig, 1960; Vietinghoff-Riesch, 1960). Thus, dormice synchronize lactation, which is distinctly later than in other hibernating mammals, with the availability of ripe seeds (Bieber and Ruf, 2004). Energy availability is limiting for successful lactation in this species. Females in good condition, with high body mass, breed earlier, while females in poor condition delay pregnancy so that lactation occurs when feeding conditions are at their best (Pilastro et al., 1994). Lactation creates extremely high energy demands, and supplemental feeding during the lactation period leads to increased growth rates of young (Zoufal, 2005; Kager and Fietz, 2009). Accordingly, heavy females produce young with a higher mean body mass at weaning, but litter size is not affected by female body mass (Pilastro et al., 1994; Kager and Fietz, 2009).

The high costs of lactation mean that, among mammals, females frequently invest more into reproduction than males (e.g., Clutton-Brock, 1991). Thus, given the relatively low energetic cost of maintaining functional testes, it seems puzzling that in dormice males forego any chances for reproduction by remaining entirely sexually inactive. In mast-failure years most males maintain regressed testes, even when some older females do reproduce, due to little chances to survive to the next year (Schlund et al., 2002; Ruf et al., 2006; Hoelzl et al., 2016b). One reason for this unusual behavior may be the fact that in mast failure years juveniles sired by reproductively active males are probably unable to acquire sufficient fat reserves prior to hibernation, which leads to negligible chances of winter survival. In addition, a long-term capture-recapture study has shown that reproduction in dormice is costly in terms of survival in both males and females, leading to the counter-intuitive pattern that survival is higher in poor years of low food abundance (Ruf et al., 2006). This effect of reproduction was found in different populations across Europe, and the negative impact of reproduction was even slightly greater in males than in females (Lebl et al., 2011a). Reduced survival probably originates mainly from the greater foraging activity and associated predation risk in reproductive years (Bieber et al., 2017; Cornils et al., 2018) but may also be caused by energetic bottlenecks. As pointed out by Fietz et al. (2004, 2005) male dormice pay a much higher energetic price for sexual competence than that caused by spermatogenesis alone. This is because testosterone production by functional testes prevents males from using short bouts of torpor during the summer-active season (Jallageas and Assenmacher, 1983). This pathway of energy saving is therefore restricted to non-reproductive years (Fietz et al., 2004, 2010; Bieber et al., 2017). In the early summer of reproductive years, males actually lose body mass, likely due to high costs of competition for mates (Bieber, 1998; Pilastro et al., 2003; Ściński and Borowski, 2008). Thus, in years without beechnuts as a high-quality food resource, males might simply not be able to cope with these costs and fail to develop functional testes (Fietz et al., 2005).

The regulation of reproduction in dormouse is, however, far from just an on/off switch linked to mast seeding. Particularly interesting, in this context, are intermediate years in which only a fraction of females have litters. A detailed study on reproductive decisions under these circumstances (Lebl et al., 2011b) confirmed that adult animals are more likely to have offspring in any particular year than yearlings (see also **Figure 1**), but that body mass is not a crucial factor determining whether a female will reproduce, even in an intermediate year (Bieber, 1998; Pilastro et al., 2003; Fietz et al., 2005). This independence of reproductive investment from energy stores makes dormice typical income breeders (Stephens et al., 2014; Williams et al., 2017). A decisive environmental factor affecting reproduction turned out to be the age of the beech forest in the home ranges of females. The proportion of females reproducing strongly increased with the mean age of trees, reaching close to 100% at a tree age of ≥80 years (Lebl et al., 2011b). Arguably, this is because older trees produce more seeds, and they also produce seeds more frequently (Nilsson and Wästljung, 1987; Yamauchi, 1996; Genet et al., 2009). Somewhat surprisingly, a high proportion of beech compared with other tree species had either no impact on reproduction (in most years), or even a negative effect on the number of females reproducing during a year with low-intermediate beechnut availability. This suggested that, in years with little seed production, areas with low beech density may provide better resources for reproduction, because there are often alternative foods such as fruits, berries, or other seeds (Lebl et al., 2011b). As mentioned before, dormice populations may persist and reproduce at low rates even in the absence of large seed trees (Bieber and Ruf, 2009a). In view of these facts it may be less surprising that an environmental niche factor analysis revealed that dormice actually avoid areas with high beech tree density, but instead prefer areas with a relatively high proportion of coniferous trees (Cornils et al., 2017). Conifer cones and leaves are another alternative food source for edible dormice (e.g., Santini, 1978). Coniferous trees also have fluctuating mast seeding events, but in the distribution range of dormice there are often several conifer species, which show only moderate synchrony of seeding (Koenig and Knops, 1998). Consequently, their collective seed production is much more stable over time than that of beech (Cornils et al., 2017). Accordingly, areas dominated by conifers can sustain the same densities of dormice as in beech forests, and dormice in more diverse mixed beech and conifer forests have even higher survival rates (but lower reproductive output) than dormice in beech forests (Schlund et al., 2002; Ruf et al., 2006).

Local resource availability for the investment into reproduction is, however, only one of the factors that determines habitat suitability for dormice. While coniferous forests interspersed with mature beech may be optimal in terms of reproductive success, there also other aspects that determine habitats, particularly predation risk. Edible dormice most frequently inhabit forests with closed canopies and stands with mid-canopies and high vertical stratification (Cornils et al., 2017), features that are avoided by their main predators, aerial-hunting owls (Hunter et al., 1995). Hence, it appears that suitable habitats are mainly characterized by predator avoidance, because vertical stratification of forests means a lower mean age of trees and hence suboptimal resource availability (Lebl et al., 2011b; Cornils et al., 2017). Since dormice are able to escape from predation almost completely by retreating to underground burrows, it seems logical that they should do so even in summer in years of reproduction skipping, when there is no good reason to stay active. As it turns out this is exactly what dormice do, at least the fraction of individuals that can afford to do so energetically.

Dormice with a high body mass at emergence can, after an active period of only a few weeks, retreat to hibernacula again in June (Hoelzl et al., 2015). They immediately commence hibernation (or technically "estivation," during the summer) until the next spring, for a total duration of up to 11.4 months (**Figure 4**). Thus, the animals briefly emerge after an 8 months hibernation period only to enter hibernation for almost another year, resulting in 19 out of 21 months spent hibernating (Bieber and Ruf, 2009b). Because dormice are fasting during hibernation, this behavior requires extremely large fat stores, and was observed only in approximately 50% of the animals. Thus, the onset of estivation occurred progressively earlier as body mass increased (Hoelzl et al., 2015). However, dormice estivate as deep as >60 cm below ground (Hoelzl et al., 2015), i.e., at relatively low T_a during summer, which limits arousal frequency und thus energy expenditure.

A hibernation duration of more than 11 months, which is longer than in any other free-living mammal, may be the most effective means of predator avoidance (Turbill et al., 2011; Geiser and Brigham, 2012). Importantly, estivation is not induced by energetic constraints in spring (Bieber and Ruf, 2009b) but appears to be an example of anticipation of the lack of future resources for successful reproduction. In other words, dormice respond to pulsed resources by making use of equally pulsed periods of extensive hypometabolism and cryptic behavior. This adaptation seems to occur in various populations. For instance, in an 18-year study in England, Trout et al. (2015, 2018) found that of those animals known to be alive, ca. 90% were captured in nest boxes in reproductive years but only 10–35% in non-reproductive years, presumably because the unseen animals were estivating. In fact, dormice routinely appear to vanish during summer, as indicated by low recapture rates (of living animals) in non-reproductive years in different populations across Europe (Lebl et al., 2011a).

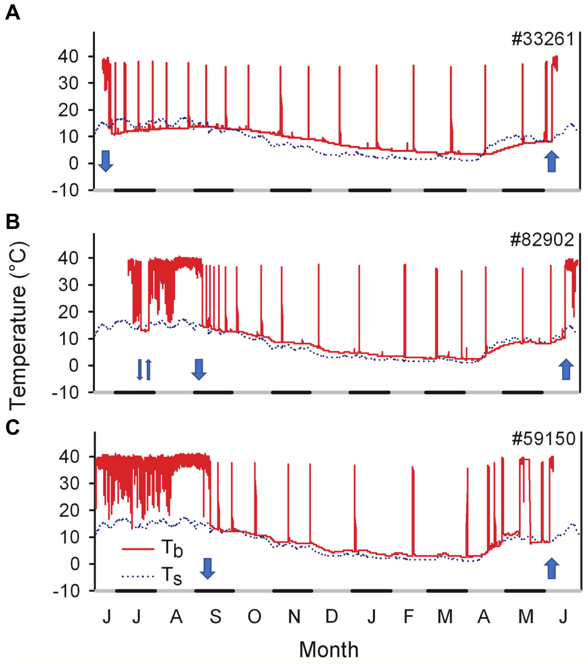

FIGURE 4 | Year-round records of core body temperature (T_b, solid lines) in three free-living edible dormice in 2012/2013. **(A)** Example of an animal showing an early onset of hibernation in June and staying in hibernation for 331 days. **(B)** An animal using multiday torpor during the active season in July. **(C)** An individual showing a long active season, entering hibernation in September. Torpor T_b during hibernation and summer dormancy was close to soil temperature (T_s, dotted lines). The arrows indicate retreat into (arrow down) and emergence from (arrow up) underground hibernacula. Modified from Hoelzl et al. (2015).

RAPID FLUCTUATIONS

Even if a substantial fraction of animals are dormant in non-reproductive years, the individuals remaining active, and almost all dormice in reproductive years, will be exposed short-term changes in external conditions. These rapid changes, particularly due to weather, are the third type of environmental fluctuations that may induce physiological and behavioral adjustments. Among rodents, responses of activity levels, thermoregulation, or social behavior to weather conditions are common (e.g., Kenagy, 1973; Vogt and Lynch, 1982; Gilbert et al., 2010; Williams et al., 2014b; Wróbel and Bogdziewicz, 2015).

Currently, there are no detailed studies of activity patterns by edible dormice under changing weather conditions. However, in the closely related common dormouse (*M. avellanarius*) activity is strongly influenced from night to night, mainly by T_a. Lower T_a has the effect of shortening activity, and cold nights may promote diurnal activity on rare occasions. There is also a weaker negative impact of rainfall on activity length in spring (Bright et al., 1996). Bright et al. (1996) suggested that hibernators are able to greatly reduce foraging activity because they can compensate for the ensuing reduced energy intake by facultative torpor.

This would also apply to the edible dormouse, who may not only exhibit hibernation and estivation but also short bouts of torpor (<24 h, **Figures 4B,C**). These bouts are often called daily torpor (e.g., Wilz and Heldmaier, 2000) although it is not clear if these short bouts in hibernators are physiologically equivalent to those in daily heterotherms. Metabolic rates during short bouts of torpor are close to hibernation metabolic rates and a magnitude lower than typically recorded during daily torpor in daily heterotherms (Wilz and Heldmaier, 2000; Ruf and Geiser, 2015). This contributes to large energy savings via short bouts of torpor, which can reach up to 80%, depending on its duration (3–21 h; Wilz and Heldmaier, 2000). In free-living dormice, short torpor is largely restricted to the photophase and on average lasts ~7.5 h (Hoelzl et al., 2015). Torpor bouts mostly occur early in the active season, predominantly on cool days (**Figures 4B,C**; Hoelzl et al., 2015; Bieber et al., 2017). However, during cold spells in spring or early summer, dormice can also prolong hypometabolism and undergo multiday bouts of torpor, i.e., estivate (**Figure 4B**). Minimum T_b during multiday torpor frequently falls well below T_a, indicating that these episodes occur almost certainly in underground burrows (Hoelzl et al., 2015).

As mentioned before, the use of torpor seems to be incompatible with reproduction in dormice (Fietz et al., 2010), although not in all mammals (McAllan and Geiser, 2014). Consequently, short bouts of torpor occur almost exclusively in non-reproductive years and only rarely in mast years, prior to the reproductive season (Bieber et al., 2017). There may be a link in the physiological regulation of torpor and locomotor activity, because in dormice there is a direct correlation between frequent torpor bouts and low activity, and the animals are much more active in reproductive years (Bieber et al., 2017). It may seem that this association merely reflects the fact that dormice may use torpor to compensate for low foraging duration and hence reduced food intake, induced by low T_a (Bright et al., 1996). However, daily heterotherms may have low rates of activity during periods of high torpor use even when food is abundant (Ruf et al., 1991; Ruf and Heldmaier, 2000). Arguably, this is because frequent torpor lowers total daily energy requirements, which in turn lowers the need for foraging. It is this complementary reinforcement which explains that energy savings via torpor and the associated reduction in activity are much higher than to be expected from hypometabolism in torpor alone (Ruf et al., 1991; Ruf and Heldmaier, 2000).

In dormice, any reduction in locomotor activity will significantly reduce energy expenditure, even independent of torpor use. In mast seeding years, dormice cease the use of short torpor with the onset of reproduction, and are highly active during the late summer season. While foraging at night under these conditions, both sexes reach core T_bs exceeding 40°C for several hours (Bieber et al., 2017). This indication of high foraging effort is most pronounced in lactating females, and is typical of the expensive lifestyle of arboreal mammals (Karasov, 1981). Any reduction of these costs of activity, as in non-reproductive years (Bieber et al., 2017), should result in profound energy savings.

Short torpor, brief episodes of estivation, and adjustments of activity are not the only responses of edible dormice to fluctuating environmental conditions. It has long been known that dormice inhabiting nest boxes (as a substitute for natural tree holes), while often solitary, aggregate in groups of up to 24 adults in a single box (Gatter and Schütt, 1999). We recently found that these groups, in that study consisting of up to 16 dormice, are aggregating predominantly in response to cold in the previous night (**Figure 5**; Ruf and Bieber, 2020). Also, these short-term aggregations during the daily resting phase are mainly formed by smaller animals, often yearlings, which have higher rates of heat loss due to a high surface to volume ratio (Fietz et al., 2010; Ruf and Bieber, 2020). Thus, the principal function of this group formation is social thermoregulation, i.e., huddling (review in Gilbert et al., 2010).

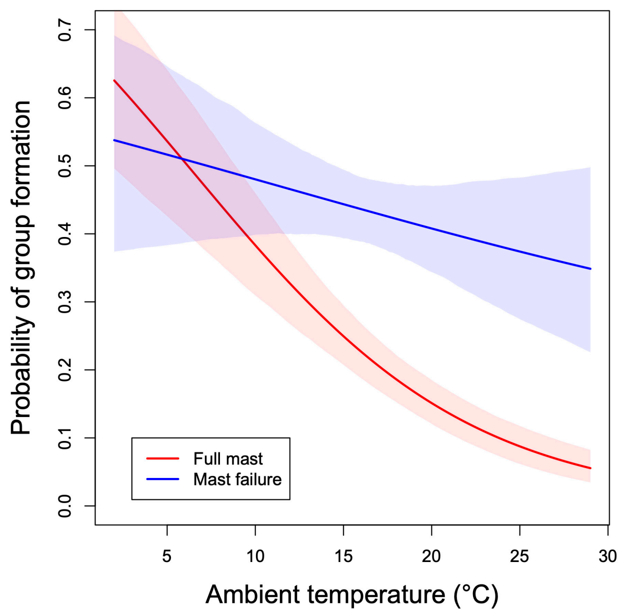

FIGURE 5 | Probability of group formation as a function of ambient temperature during the past 24 h in edible dormice. Groups consisted of 2–16 adults and yearlings of both sexes. Predictions ± 95% credible intervals from a Bayesian multilevel model for years of full mast seeding (red) and mast failure years (blue) of beech trees. Data from a 13-year mark-recapture study, Austria. Recomputed from data in Ruf and Bieber (2020).

Not unexpectedly, the proclivity for huddling in dormice varies between mast seeding and mast failure years. In mast seeding/reproductive years, both sexes appear reluctant to aggregate and form huddles only at low T_a (**Figure 5**). However, their capability to huddle even in reproductive years indicates that elevated levels of reproductive hormones do not prevent this behavior. Aggregations during the daily resting phase come at the cost of having to share food resources during foraging at night. Indeed, at least in beech forests home-ranges in both sexes are relatively small, except for lactating females (ca. 0.8 ha, Hönel, 1991). Apparently, the high energy demands of competition for mates and investment into reproduction select against sharing of food resources in reproductive years. Lactating females in particular never seem to share nest boxes with males, although they can occasionally communally nest with related females (Pilastro, 1992; Ruf and Bieber, 2020). In contrast, in mast-failure years the tendency for huddling is higher, even at warmer T_as (**Figure 5**).

Dormice mitigate the consequences of resource competition by preferentially huddling with relatives. This is achieved by 'family reunions' of mothers and siblings, or siblings only, which join the same groups as yearlings and adults, suggesting that this behavior has evolved through kin selection (Ruf and Bieber, 2020). Costs of resource competition also appear to be alleviated by a surplus of males, which probably have lower energy demands than reproducing females (Ruf and Bieber, 2020). Remarkably, despite the potential of the aggression-promoting effects of testosterone, aggregations include males with fully developed testes (Fietz et al., 2004, 2010; Ruf and Bieber, 2020). As pointed out by Fietz and colleagues, for sexually active males that do not use torpor, social thermoregulation may be the only response to cold left (Fietz et al., 2010). Interestingly, the use of huddling, predominantly in mast failure years, means that dormice show strong year-to-year shifts in their degree of sociality. These alterations seem rare but have been also reported in the sister-clade of the dormice, the Sciurids, in which females of otherwise 'asocial' species may communally nest outside the reproductive season (Williams et al., 2013). Of course, social thermoregulation and other tactics to adverse environmental conditions are by no means mutually exclusive. For instance, dormice may use short bouts of torpor while resting in huddling groups (Ruf and Bieber, 2020), and these behavioral responses can facilitate each other, which increases their energetic efficiency (Ruf et al., 1991; Jefimow et al., 2011; Eto et al., 2014).

SYNOPSIS: COMPARATIVE ASPECTS

The primary responses of edible dormice to seasonal changes in food availability and climate, namely, seasonal reproduction followed by energy storage, gonadal involution and hibernation are common among mammals. At least 93 species of mammals from 11 orders are known to hibernate (Ruf and Geiser, 2015) and the cessation of energy-demanding reproductive processes during the hibernation season is the rule (but see Morrow and Nicol, 2009). However, the extent of hibernation- use in dormice is extremely large. This concerns both their average (8 months) and maximum hibernation duration (>11 months), which is significantly longer than, for instance, among the Marmotini (Williams et al., 2014a). Arguably, these characteristics are mainly due to the high energy density of seeds [7.0–7.9 kcal/g dry mass (dm); Grodziński and Sawicka-Kapusta, 1970], the preferred food of dormice in reproductive years. It is this high-caloric food which enables rapid fattening as well as extremely fast growth of young, factors that allow for the production of single litter very late in summer, compared with other hibernators (Bieber and Ruf, 2004). In those hibernators predominantly feeding on herbage during the reproductive season [4.0–4.6 kcal/g dm; (Hutton, 1962)], such as several ground squirrels and marmots, juvenile growth is much slower. This slow development favors reproduction early in the season and thus constrains the hibernation duration.

The second source of environmental variation affecting dormice, mast-seeding of trees with large changes in energy availability, creates strong pulses in reproductive output. This response is not uncommon, however. Other seed predators such as American and Eurasian tree squirrels (*Tamiasciurus hudsonicus*) also anticipate resource pulses and increase reproductive output before a masting event, whereas another squirrel (*Sciurus vulgaris*) increases reproduction following mast seeding of Norway spruce (Selonen et al., 2015). Responses of dormice most closely resemble those of Eastern chipmunks (*Tamias striatus*) which reproduce intensely prior to American beech mast [*Fagus grandifolia*; (Bergeron et al., 2011)]. Strikingly, just like dormice, chipmunks were found to interrupt aboveground activity for 9–11 months when mast was not available (Munro et al., 2008). However, while Eastern chipmunks are hibernators, this inactivity may not reflect aestivation, because large food hoards enable this species to cease foraging even without the use of torpor (Humphries et al., 2003; Munro et al., 2008).

Pulsed resources do no only affects graminivores. For instance, omnivores, such as the wild boar, have increased reproductive output after mast seeding of beech or oak (Bieber and Ruf, 2005). Similarly, the Kangaroo rat (*Dipodomys merriami*), which requires green plants for reproduction, is a "pulse matcher" that responds directly to the occurrence of pulses of food production (Kenagy and Bartholomew, 1985). Insectivorous bats such- as the lesser mouse-eared bat (*Myotis blythii*) respond to years with superabundance of cockchafers in spring by advancing parturition, and by delaying puberty, compared with their sibling species *Myotis myotis*, which exploits this food resource less (Arlettaz et al., 2017). Insectivores, especially bats, are also the largest group among the mammalian hibernators (45 out of 93 species; Grizmek, 1997; Ruf and Geiser, 2015). Currently it is apparently unknown, however, whether any pulsed resource trackers among these species use extended hibernation during years of low food abundance. Pulsed resources also affect reproduction in insectivorous birds (Ostfeld and Keesing, 2000; Drever et al., 2009), but there are no interactions with hibernation, because there is only one bird species known to hibernate, i.e., to use multiday torpor (Jaeger, 1949). However, many birds use daily torpor in both winter and summer (Geiser, 2010; Ruf and Geiser, 2015), and torpor frequency in birds can be strongly increased by low arthropod abundance (e.g., Doucette et al., 2012). Thus, it seems that a variety of mammals and birds are capable of flexibly using heterothermy to adjust energy expenditure to variations in food availability, far beyond responding only to the typical scarcity of food during winter in temperate and arctic zones. Since this use of heterothermy serves to adjust energy turnover to maximize fitness, it may be viewed as a flexible life-history trait, not just in dormice. Arguably, species adapted to pulsed resources that are synchronized over large geographical zones, such as dormice or chipmunks, are only the most conspicuous examples for these effects, because entire populations respond simultaneously. In other species, effects of reproduction on the timing of hibernation, and its use to adjust energy expenditure to reproductive effort, may occur only locally and/or affect individuals differently (e.g., Michener, 1983; Lehmer et al., 2006; Millesi et al., 2008; Zervanos et al., 2010; Siutz et al., 2016).

The responses of edible dormice to short-term variation in weather conditions, namely short torpor and huddling, are also widespread among endotherms (reviews in Gilbert et al., 2010; Ruf and Geiser, 2015). One interesting avenue of future research in this context would be the joint energetic effects of these responses, in species like dormice that use them simultaneously (cf. Jefimow et al., 2011). Taken together, it seems that edible dormice use a variety of strategies to respond to environmental fluctuations, like hibernation, aestivation, anticipatory reproduction and reproduction skipping, as well as short torpor and huddling, which are not uncommon adaptations among endotherms. The only unusual aspect seems to be their combined occurrence in a single species. One of the most pertinent questions for future research on dormice and other pulsed resource consumers will be the effects of global climate change on mast frequency and the long-term consequences for both trees and seed predators.

AUTHOR CONTRIBUTIONS

TR and CB conceived of this review and TR wrote the first draft. CB commented, critically revised, and edited the manuscript. TR and CB read and approved the final version of the manuscript.

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REVIEW
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Seasonal Expression of Avian and Mammalian Daily Torpor and Hibernation: Not a Simple Summer-Winter Affair[†]

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Daily torpor and hibernation (multiday torpor) are the most efficient means for energy conservation in endothermic birds and mammals and are used by many small species to deal with a number of challenges. These include seasonal adverse environmental conditions and low food/water availability, periods of high energetic demands, but also reduced foraging options because of high predation pressure. Because such challenges differ among regions, habitats and food consumed by animals, the seasonal expression of torpor also varies, but the seasonality of torpor is often not as clear-cut as is commonly assumed and differs between hibernators and daily heterotherms expressing daily torpor exclusively. Hibernation is found in mammals from all three subclasses from the arctic to the tropics, but is known for only one bird. Several hibernators can hibernate for an entire year or express torpor throughout the year (8% of species) and more hibernate from late summer to spring (14%). The most typical hibernation season is the cold season from fall to spring (48%), whereas hibernation is rarely restricted to winter (6%). In hibernators, torpor expression changes significantly with season, with strong seasonality mainly found in the sciurid and cricetid rodents, but seasonality is less pronounced in the marsupials, bats and dormice. Daily torpor is diverse in both mammals and birds, typically is not as seasonal as hibernation and torpor expression does not change significantly with season. Torpor in spring/summer has several selective advantages including: energy and water conservation, facilitation of reproduction or growth during development with limited resources, or minimisation of foraging and thus exposure to predators. When torpor is expressed in spring/summer it is usually not as deep and long as in winter, because of higher ambient temperatures, but also due to seasonal functional plasticity. Unlike many other species, subtropical nectarivorous blossom-bats and desert spiny mice use more frequent and pronounced torpor in summer than in winter, which is related to seasonal availability of nectar or water. Thus, seasonal use of torpor is complex and differs among species and habitats.

Keywords: daily torpor, energy expenditure, heterothermy, hibernation, reproduction, season, torpor cost-benefit

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INTRODUCTION

The climate and weather of most geographical regions change substantially with season. This reflects to a large extent the yearly cycle of the rotating earth with its tilted axis around the sun, the distance from the equator, the elevation of the terrain, but also a number of specific local environmental factors. The resulting most obvious physical changes of seasonal environmental variables include ambient temperature (T_a) and day-length, but often also a change in precipitation or wind speed. The change in T_a is crucial to many organisms, as it strongly affects their environment and their bodily functions, such as energy expenditure, thermoregulation, locomotion, reproduction and growth.

Especially endothermic (capable of producing substantial amounts of heat internally) birds and mammals are strongly, but indirectly, affected by T_a (Withers et al., 2016). Endotherms can regulate a high and constant body temperature (T_b) over a wide range of T_a to a large extent by fine adjustments of heat production below the thermo-neutral zone (TNZ) and heat loss within and above the TNZ. In the TNZ the metabolic rate (MR) in normothermic (constant high "normal" T_b) and resting endotherms can be minimal or "basal" (BMR). Above the TNZ cooling of the body is accomplished by evaporation of water, which is facilitated by sweating, increased ventilation or postural changes, and together with an increase in T_b results in an increase in MR. Below the TNZ metabolic rate is inversely related to T_a, because heat loss is a function of the T_b–T_a differential. To achieve this, the animals must produce large amounts of internal heat by shivering or non-shivering thermogenesis (Nowack et al., 2017a) to replace the heat lost from the body to the environment (Withers et al., 2016). As T_a is usually lower at night than during the daytime, mammals, most of which are nocturnal, are faced with an extra challenge, but to some extent can use heat generated by activity for thermoregulation. In contrast, most birds are diurnal and have to produce sufficient heat while resting during the night if they are to remain normothermic, although some of the birds to be discussed here are also nocturnal.

The majority of birds and mammals are small and because surface area and body mass or body volume are inversely related, heat loss is also strongly affected by body size (Withers et al., 2016). This can be problematic for small birds and mammals because compensation for heat loss by internal heat production is energetically expensive and requires the intake of large amounts of food to fuel the high metabolism. The time of year that is of special concern in many, but not all, regions is of course winter when T_a is low together with a low availability of food. To a large extent because of such energetic challenges many species that can fly (birds and bats) and can cover large distances fast and energetically cheaply (Tucker, 1975) avoid these conditions and migrate to more benign areas often over long distances. Small non-volant species cannot move over long distances because their locomotion is slow and energetically expensive (Tucker, 1975). Therefore sedentary species have to deal with thermal conditions and food availability in or near their usual home range by using other behavioral and physiological approaches instead (Körtner et al., 2000).

Many small endothermic mammals and birds are therefore not permanently homeothermic (have a constant T_b), but rather are heterothermic (have a fluctuating T_b) and use torpor for energy conservation, most typically during times of food shortage and/or cold exposure (Kayser, 1961; Dawson and Hudson, 1970; Lyman et al., 1982; Reinertsen, 1983; Boyer and Barnes, 1999; McKechnie and Lovegrove, 2002; Schleucher, 2004). However, torpor has many other selective advantages including water conservation, enabling reproduction and development, dealing with storms, fires, heat waves, floods or increased predator presence (Schmid and Speakman, 2009; Nowack et al., 2017b, 2020; Barak et al., 2019; Renninger et al., 2020). Torpor is characterized by substantial and controlled reductions in MR (often by 50–95%) and T_b (often by ~5–35°C) and may last for a part of the day (daily torpor in the daily heterotherms), or for a number of days up to weeks (hibernation or multiday torpor in the hibernators), but multiday torpor is interrupted by periodic arousal to normothermic T_b in most. Daily torpor and hibernation in most heterotherms differ both ecologically and functionally. Hibernators often show extensive fattening before the torpor period, by ~35% in dormice (*G. glis*; Fietz et al., 2003; Lebl et al., 2011; Bieber et al., 2014) or fewer store food (Humphries et al., 2003) and many species show no or only limited foraging during the hibernation season. Hibernators reduce T_b from ~38 to ~5°C (many to between 0 and 5°C) and the torpor MR (TMR) to ~5% of BMR on average (Ruf and Geiser, 2015). In daily heterotherms, the T_b falls to ~18°C and the TMR is ~30% of the BMR on average (Ruf and Geiser, 2015; Shankar et al., 2018). Daily heterotherms may lose body mass or fatten only little before the torpor season and torpor is interrupted by periodic, often daily, foraging. Importantly, even during the state of torpor when the TMR is at a minimum, animals remain endothermic and can control their T_b above a critical minimum (Hainsworth and Wolf, 1970; Heller and Hammel, 1972), likely to avoid tissue or organ damage that seems to occur well above 0°C in many species, and, in species exposed to sub-zero T_as, to avoid freezing (Barnes, 1989).

As it is sometimes claimed that there are no distinct patterns of torpor, but rather a continuum of variables when comparisons are based on T_b measures such as the 'heterothermy index' (e.g., Boyles et al., 2013), I attempted to use this approach initially for analyzing the data. However, since the heterothermy index failed to differentiate between deep, short and long, shallow torpor bouts resulting in the same heterothermy index for entirely different patterns of torpor (**Figure 1**), this method was not further pursued and the traditional approach using physiological minima or maxima expressed by a species was used for classification and analyses instead (Brigham et al., 2011; Ruf and Geiser, 2015).

Both daily torpor and hibernation, as stated above, are typically interrupted by energetically costly periodic arousals using endogenous heat production, and maintenance of normothermic T_b for periods usually lasting for several hours (Boyer and Barnes, 1999; McKechnie and Lovegrove, 2002; Ruf and Geiser, 2015). However, despite periodic arousals and the long duration of the hibernation season for about two-thirds of a year, energy expenditure during the hibernation season was only 13-17% of the annual energy expenditure in ground squirrels (*Callospermophilus* syn. *Spermophilus saturatus*) (Kenagy et al., 1989). In pygmy-possums (*Cercartetus nanus*) energy expenditure during a one-year hibernation season in captivity was only ∼4% of the predicted field metabolic rate (Geiser, 2007).

Whereas daily heterotherms often use the normothermic periods between torpor bouts for foraging and feeding (Körtner et al., 2008) in hibernators the physiological requirement of periodic arousals to normothermic T_b is still not fully understood. However, as outlined below, it is likely linked to restorative functions to counteract some dysfunction that occurred during torpor (Willis, 1982; Geiser et al., 1990; Daan et al., 1991; Prendergast et al., 2002). Periodic rewarming from hibernation seems to be related to the usually low T_b experienced during deep torpor because in a few species periodic rewarming does not occur during hibernation at high T_b (Lovegrove et al., 2014; but see Liu and Karasov, 2011), or periodic rewarming is partly or entirely passive (Dausmann and Warnecke, 2016), which seems to suffice. With regard to migration, although it is used to avoid adverse conditions in many flying species, migration does not preclude torpor as it can have an important role during migration stopovers in birds as well as in bats to save energy and maximize energy availability for the next flight (Carpenter and Hixon, 1988; Hiebert, 1993; Wojciechowski and Pinshow, 2009; McGuire et al., 2014).

Torpor is extremely diverse and occurs in all mammalian subclasses and in at least ten avian orders (McKechnie and Lovegrove, 2002; Ruf and Geiser, 2015). Torpor also has been described in all geographic regions of the world and these differ substantially with regard to seasonal challenges. Whereas temperate and high latitude/altitude regions are typically characterised by warm T_as in summer and often high primary productivity, T_as in winter are low resulting in little or no primary productivity, tropical areas may remain rather warm in winter, but often show strong seasonal changes in rainfall with almost all precipitation in summer and none in winter. In subtropical areas the high summer heat may limit plant productivity and, for example nectar production, can be much higher during the mild winters (Ford, 1989). In deserts T_as are often too hot and/or precipitation too low in summer for significant plant growth, whereas winters can be rather mild during the day at least in deserts not too far from the equator, as for example the Australian deserts. The seasonal change in photoperiod, a reliable environmental signal for seasonal change in physiology, also differs enormously between high and low latitudes. Such regional differences are reflected in the seasonal expression of torpor.

My review aims to summarize the different seasonal patterns of torpor use of endotherms and relate them to their environment and reproduction. Other aspects of the seasonal change in torpor expression have been reviewed recently with regard to seasonal energy use (Kenagy, 1989) circadian and circannual rhythms (Körtner and Geiser, 2000b) or its neural and endocrine control (Jastroch et al., 2016). I tried to use data on free-ranging animals and animals kept under natural photoperiod and T_a fluctuations or captured in the wild, but data on seasonal torpor under laboratory conditions, if they seemed relevant, are also reported. Overall, much fewer quantitative data are available for summer than for winter. Known torpor occurrence for the four seasons in hibernators and daily heterotherms were analyzed using Chi-Square tests. Seasons are defined as spring (March to May), summer (June to August), autumn (September to November), and winter (December to February) for the northern hemisphere and the reverse for the southern hemisphere. The data on seasonal torpor expression are also used to examine hypotheses in relation to the widely assumed clearly different seasonal summer and winter phenotypes for heterotherms in general (e.g., Williams et al., 2005) and whether the observed seasonal torpor expression can be explained by or supports the cost-benefit approach outlined by Humphries et al. (2003).

Sections are generally summarized under different orders or sub-classes, but since rodents were the first group that was examined in detail with regard to seasonal hibernation in the field, they are covered first. The tables are presented in the usual taxonomic order. As most quantifications of torpor were based on T_b measures, a reduction of T_b by > 5°C below the normothermic resting T_b was used to define torpor (example shown in **Figure 1**); the time when T_b remained > 5°C below resting T_b was defined as torpor bout duration (TBD) (Ruf and Geiser, 2015). Hibernators are defined as species that can express multiday torpor of > 2 days, whereas daily heterotherms are defined as species expressing daily torpor exclusively under all thermal, environmental and nutritional conditions. "Shallow" torpor describes a reduction of T_b by 5–10°C below normothermic resting T_b. When only MR measures were available, a reduction of MR by > 25% below the RMR at the same T_a was used to define torpor (Hudson and Scott, 1979).

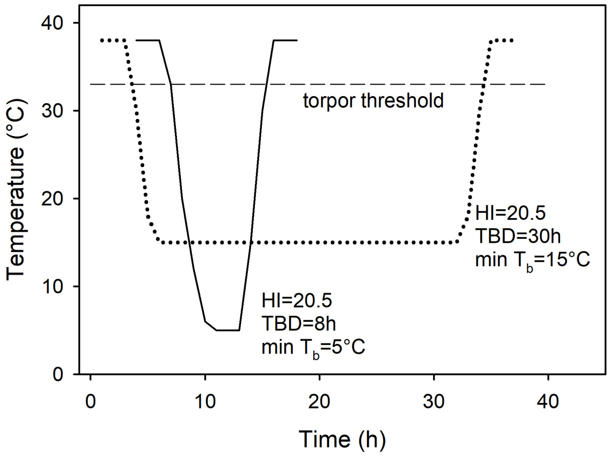

FIGURE 1 | Two constructed, but realistic bouts of torpor, one deep and brief (solid line), the other shallow and long (dotted line) showing the change of body temperature over time and the respective heterothermy index (HI). Although the torpor bouts differ enormously with regard to the minimum T_b and the torpor bout duration (TBD), the HI is identical. Also shown is the torpor threshold (broken line), in this example at T_b = 33°C because the normothermic resting T_b = 38°C.

HIBERNATION

It has been known for centuries that some mammals disappear in winter and that they hibernate during this time. Much of the original work was done in northern Europe or America with some quantitative work appearing in the early eighteen-hundreds Hall (1832) or even before (see Kayser, 1961; Mrosovsky, 1971; Lyman et al., 1982). Hibernation is especially obvious in diurnal sciurid rodents, such as ground squirrels, chipmunks and marmots, which disappear into burrows around autumn and re-appear in spring and their seasonal use of torpor can be quantified to a large extent by observation or trapping. It is therefore of little surprise that the widely held view of seasonal expression of torpor, or specifically hibernation (from Latin “hibernare” to spend the winter) is one of torpor use in late autumn, winter and early spring, unlike the rest of the year, which is supposed to be devoted to activity and reproduction. Obviously, the term hibernation has a seasonal connotation implying that it only occurs in winter, but, as we will see below, hibernation is only rarely restricted to winter and may in fact last for much of the year in some species and under certain circumstances (**Figure 2**).

Yearlong Hibernation

The most extreme expression of hibernation is known for three unrelated mammals, phylogenetically separated for > 120 Million years, the marsupial eastern pygmy-possum (*Cercartetus nanus*) from Australia and the dormice (*G. glis* and *Eliomys quercinus*), rodents from Eurasia (**Figure 2** and **Table 1**). These hibernators do not only hibernate in winter, but, under laboratory conditions, can do so for an entire year. In captivity the pygmy-possums managed to hibernate at T_a 7°C for over 12 months, relying entirely on stored body fat for energy expenditure (Geiser, 2007). Edible dormice (*G. glis*) bought from a breeder in France hibernated in a Canadian cold room at T_a 5°C for up to a year, but since body mass cycles were investigated it appears that food was available and animals may have fed (Mrosovsky, 1977). In another study, captive non-reproductive *G. glis* hibernated for up to 11 months/year, and, as animals had access to food and were in good condition, the prolonged underground hibernation was interpreted as having evolved as means for predator avoidance in the wild (Bieber and Ruf, 2009). Free-ranging non-reproductive *G. glis* also can hibernate for up to 11 months (Hoelzl et al., 2015). Captive garden dormice (*E. quercinus*), also purchased from France, expressed torpor throughout the year when held in Holland at T_a 12°C, but the TBD was shorter in summer than in winter (Daan, 1973).

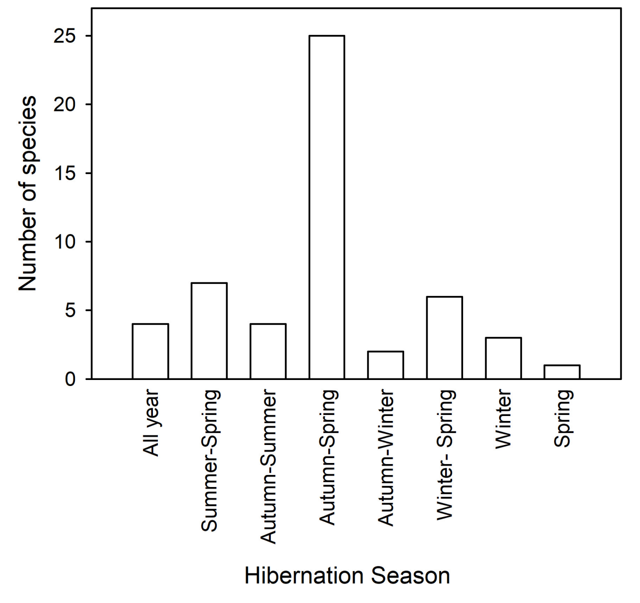

FIGURE 2 | The duration of the hibernation season and the number of species expressing multiday torpor during each season is shown. The data and references are listed in **Table 1**.

It could be argued that yearlong hibernation or at least the yearlong use of torpor also occurs in long-eared bats (*Nyctophilus* spp.) and perhaps other bats as they can display multiday torpor throughout the year in the wild including during the period of reproduction (see below). In mouse-eared bats (*Myotis myotis*) torpor expression was largely independent of season but rather was affected by T_a (Wojciechowski et al., 2007). Moreover, in several species held in captivity (female marmots *Marmota flaviventris*, ground squirrels *Urocitellus* syn. *Spermophilus beldingi*, jumping mice *Zapus princeps*), hibernation could be artificially extended by continued cold exposure to > 300 days (French, 1985). These findings suggest that hibernation for up to one year may be functionally possible in many hibernators.

Seasonal Hibernation

Mammals

Rodents

A common seasonal pattern of hibernation in the northern hemisphere is torpor expression from late summer/autumn to spring. It occurs and has been investigated in detail in sciurid rodents such as ground squirrels, chipmunks and marmots (Sciuridae), dormice (Gliridae) and hibernating hamsters (Cricetidae).

The classic pattern of seasonal hibernation in free-ranging mammals was first quantified using temperature-telemetry for Richardson’s ground squirrels (*Urocitellus* syn. *Spermophilus richardsonii*) near Edmonton, Canada (Wang, 1978). The hibernation season in most individuals commenced in mid-July in adults, 2 months later in juveniles and was terminated by both groups in mid-March. Torpor in *U. richardsonii* was characterized by minimum T_bs of ∼2°C and TBDs of 10–20 days in mid-winter in the wild, but shorter in captive individuals (Wang, 1978). More specific data, especially on sexual differences, have been provided for this species by Michener (1992) and confirm that in this ground squirrel hibernation in the wild lasts from summer until spring (**Table 1**). Similar patterns of seasonal torpor expression (**Table 1**) have been observed in golden-mantled ground squirrels (*C. lateralis* and *C. saturatus*) (Kenagy et al., 1989; Healy et al., 2012), Columbian ground squirrels (*Urocitellus* syn. *Spermophilus columbianus*) (Young, 1990) and Anatolian ground squirrels (*Spermophilus*

TABLE 1 | The hibernation season when multiday torpor (>2 days) can be used by birds and mammals.

All year
Eastern pygmy possum, *Cercartetus nanus* (Geiser, 2007)
Long-eared bat, *Nyctophilus geoffroyi* (Turbill et al., 2003; Turbill and Geiser, 2008)
Edible dormouse, *Glis glis* (Mrosovsky, 1977; Hoelzl et al., 2015)
Garden dormouse, *Eliomys quercinus* (Daan, 1973)
Summer to spring
Echidna, *Tachyglossus aculeatus* (Grigg et al., 1989; Nicol and Andersen, 2007)
Anatolian ground squirrel, *Spermophilus xanthoprymnus* (Kart Gür et al., 2009)
Arctic ground squirrel, *Urocitellus/Spermophilus parryii* (Barnes, 1996)
Columbian ground squirrel, *Urocitellus/Spermophilus columbianus* (Young, 1990)
Richardson's ground squirrel, *Urocitellus/Spermophilus richardsonii* (Wang, 1978)
Golden-mantled ground squirrel, *Callospermophilus/Spermophilus saturatus* (Barnes, 1996)
Woodchuck, *Marmota monax* (Zervanos et al., 2010)
Autumn to summer
Little brown bat, *Myotis lucifugus* (Jonasson and Willis, 2012; Johnson et al., 2019)
Brandt's bat, *Myotis brandtii* (Podlutsky et al., 2005)
Hodgson's bat, *Myotis formosus* (Kim et al., 2013)
Jumping mouse, *Zapus princeps* (Cranford, 1978)
Autumn to spring
Mountain pygmy-possum, *Burramys parvus* (Körtner and Geiser, 1998)
Fat-tailed dwarf lemur, *Cheirogaleus medius* (Dausmann, 2014)
Dwarf lemur, *Cheirogaleus crossleyi* (Blanco et al., 2013; Dausmann, 2014)
European hedgehog, *Erinaceus europaeus* (Walhovd, 1979)
Algerian hedgehog *Atelerix algericus* (Mouhoub-Sayah et al., 2008)
Greater horseshoe bat, *Rhinolophus ferrumequinum* (Park et al., 2000)
Big brown bat, *Eptesicus fuscus* (French, 1985)
Indiana bat, *Myotis sodalis* (Clawson et al., 1980)
Mouse-tailed bat, *Rhinopoma microphyllum* (Levin et al., 2015)
Mouse-tailed bat, *Rhinopoma cystops* (Levin et al., 2015)
Brown bear, *Ursos arctos* (Evans et al., 2016)
Yellow-pine chipmunk, *Tamias amoenus* (Barnes, 1996)
Townsend's chipmunk, *Tamias townsendi* (Barnes, 1996)
Siberian chipmunk, *Eutamias sibiricus* (Kawamichi and Kawamichi, 1993)
Golden-mantled ground squirrel, *Callospermophilus/Spermophilus lateralis* (Healy et al., 2012)
Daurian ground squirrel, *Spermophilus dauricus* (Yang et al., 2011)
Thirteen-lined ground squirrel, *Ictidomys/Spermophilus tridecemlineatus* (Kisser and Goodwin, 2012)
Alpine marmot, *Marmota marmota* (Arnold, 1993)
Alaska marmot, *Marmota broweri* (Lee et al., 2009)
Yellow-bellied marmot, *Marmota flaviventris* (French, 1985)
Prairie dog, *Cynomys parvidens* (Lehmer and Biggins, 2005)
European hamster, *Cricetus cricetus* (Wassmer, 2004; Siutz et al., 2016)
Common or hazel dormouse, *Muscardinus avellanarius* (Pretzlaff and Dausmann, 2012)
Woolly dormouse, *Dryomys laniger* (Kart Gür et al., 2014)
Birch mouse, *Sicista betulina* (Eisentraut, 1956)
Autumn to winter
Armadillo, *Zaedyus pichiy* (Superina and Boily, 2007)
African hedgehog, *Atelerix frontalis* (Hallam and Mzilikazi, 2011)
Winter to spring
Pygmy slow loris, *Nycticebus pygmaeus* (Ruf et al., 2015)
European free-tailed bat, *Tadarida teniotis* (Arlettaz et al., 2000)
Formosan leaf-nosed bat, *Hipposideros terasensis* (Liu and Karasov, 2011)
Black bear, *Ursus americanus* (Toien et al., 2011)
Eastern chipmunk, *Tamias striatus* (Landry-Cuerrier et al., 2008)
Jerboa, *Jaculus orientalis* (El Ouezzani et al., 2011)
Winter
Poorwill, *Phalaenoptilus nuttallii* (Woods et al., 2019)
Western pygmy-possum, *Cercartetus concinnus*, field (Turner et al., 2012b)
European badger, *Meles meles* (Fowler and Racey, 1988).
Spring
Golden mole, *Amblysomus hottentottus longiceps* (Scantlebury et al., 2008)

Only species for which long-term data are available are listed and the most pronounced pattern observed for each species is reported.

xanthoprymnus) (Kart Gür et al., 2009). Hibernation from summer to spring is rather common and occurs in ∼14% of the species for which data are available (**Figure 2** and **Table 1**).

Due to the far northern distribution of Arctic ground squirrels (*Urocitellus* syn. *Spermophilus parryii*), the hibernation season is also extremely long usually lasting from August/September to April (Barnes, 1996) and deep with minimum T_bs as low as −2.9°C (Barnes, 1989; Richter et al., 2015). The beginning and end of the hibernation season differs between males and females, with males entering torpor later and emerging earlier than females to establish territories and get ready for mating (Barnes, 1996). Although the end of hibernation in March/April is inflexible in reproductive males, resulting in a potentially disastrous phenological mismatch during spring snow storms, non-reproductive males and reproductive females, after the end of the usual hibernation season, re-entered hibernation with short TBD of 1–6 days, emerged in May and thus delayed reproduction reducing the time available for growth of young and pre-hibernation fattening for the next winter (Williams et al., 2017).

In most hibernators (48% of species; **Table 1** and **Figure 2**) including thirteen-lined ground squirrels (*Ictidomys* syn. *Spermophilus tridecemlineatus*) in Michigan and Daurian ground squirrels (*Spermophilus dauricus*) in northern China, the hibernation season lasts from autumn to spring (Yang et al., 2011; Kisser and Goodwin, 2012). Thus a hibernation season lasting for more than 6 months is most common. This is also the case for European alpine marmots (*Marmota marmota*). Alpine marmots show social hibernation, periodic rewarming is highly synchronized among individuals and the degree of synchrony affects mass loss during winter (Arnold, 1988, 1993; Ruf and Arnold, 2000). When juveniles were part of a hibernating group, territorial males commenced the rewarming process earlier than juveniles, which can use the heat generated by adults for partial passive rewarming. Woodchucks (*Marmota monax*) have a very wide distribution over North America and their hibernation season differs according to latitude (Zervanos et al., 2010). In Maine (∼44°N) woodchucks hibernated from July to April, in Pennsylvania (∼40°N) from November to March and in South Carolina (∼35°N) from December to March showing a strong phenotypic flexibility or selection among populations (Zervanos et al., 2010). During a severe drought in Pennsylvania, free-ranging *M. monax* entered short bouts of torpor in August with T_b fluctuating between ∼25 and 38°C when T_a ranged from 20 to 30°C; after rainfall some individuals remained normothermic, whereas others continued to exhibit torpor (Zervanos and Salsbury, 2003). With regard to elevation, although the hibernation season in prairie dogs (*Cynomys parvidens*), which are considered to be "facultative" hibernators, lasted from autumn to spring in high and mid-elevation populations, low elevation populations terminated hibernation already in late winter, when food became available (Lehmer and Biggins, 2005).

Unlike many other hibernators, European hamsters (*Cricetus cricetus*) store food rather than mainly fat for the hibernation season (Wassmer, 2004; Siutz et al., 2016). Consequently, the gut is not reduced but rather needs to be maintained during winter (Humphries et al., 2003; Tissier et al., 2019). Nevertheless, the hibernation season is similar to that of many other rodent hibernators lasting from autumn to spring (**Figure 2**), however, the TBD is somewhat shorter (∼5 days) than in many other species (TBD often 10–20 days), and the usual sexual differences in the hibernation season are reversed, with adult males hibernating for longer than females (Siutz et al., 2016).

Chipmunks are often considered to be intermediate between food-storing and fat-storing rodent hibernators, and, although it has been claimed they rely entirely on stored food during hibernation (Humphries et al., 2003), some can also store substantial amounts of fat. In free-ranging Siberian chipmunks (*Eutamias sibericus*) measured in Hokkaido, Japan, over 7 years, hibernation commenced first in adults in September/October followed by juveniles about 1 month later; spring emergence occurred around April in adult males and in May in females and the yearly variation in the timing of hibernation reflected snow cover (Kawamichi and Kawamichi, 1993). Mortality during hibernation for all age classes was low (3.7–5.7%) whereas during the active period mortality in adults was around 50% (Kawamichi and Kawamichi, 1993). In western chipmunks, such as yellow-pine (*Tamias amoenus*) and Townsend chipmunks (*Tamias townsendi*) the hibernation season in the wild in Washington, United States, was somewhat shorter than in sympatric ground squirrels and lasted from around October/November to March (Kenagy and Barnes, 1988), but they still fall in the group with the most common hibernation season (**Figure 2** and **Table 1**). Hibernation in *T. amoenus* is rather predictable and, at least in the laboratory, is associated with substantial fattening (∼45% increase in body mass) in autumn, and animals eat little or nothing when hibernating during mid-winter despite availability of food (Geiser et al., 1990). On the other hand, in eastern chipmunks (*Tamias striatus*), studied in Quebec, torpor use differs from many other sciurids as its expression in winter even in nature can be rather variable to a large extent depending on food availability (Landry-Cuerrier et al., 2008). In good food years when many trees produced seeds, torpor in *T. striatus* was used in winter but was rather irregular and shallow (T_b often >10°C). Whereas in low-food years hibernation was characterized by a regular expression of a sequence of deep ($T_b<10$°C) and multiday torpor bouts and lasted from ∼November/December to May (Landry-Cuerrier et al., 2008). The seasonal pattern observed in *T. striatus* occurs in only ∼11% of hibernators, including mainly species from mild climates and large bears (**Table 1**). Hudson (1978) extrapolated from data on *T. striatus* that chipmunks in general may differ from " classical" hibernators by having rather high minimum T_bs of 5–7°C and because only some individuals expressed torpor in captivity. In contrast, *T. amoenus* can have very low minimum T_bs during torpor (minimum regulated T_b −1.0°C, Geiser et al., 1994) and all individuals entered torpor in captivity, although the TBD generally was somewhat shorter (∼8 days) than in sympatric ground squirrels (*C. saturatus*, ∼11 days) in mid-winter (Geiser et al., 1990). Consequently, variables of torpor measured for *T. striatus* differ from many other hibernators in an interesting way, but because of that should not be considered representative of other sciurid rodents or hibernators in general.

Overall, available information suggests that hibernation in sciurid and cricetid hibernators is a strongly seasonal event, supporting the view of different seasonal phenotypes. Nevertheless, there is some flexibility in several species, especially in juveniles, females and non-reproductive males, which can extend the hibernation season when this is required.

In the dormouse family (Gliridae) hibernation is used by several species not covered above and in several dormice torpor expression is not highly seasonal. In addition to hibernation from autumn to spring (Pretzlaff and Dausmann, 2012), hazel dormice (*Muscardinus avellanarius*) frequently expressed torpor during summer, although torpor bouts were generally brief (Pretzlaff et al., 2014). Adult male *M. avellanarius* used torpor more frequently than females during the active season in summer and pregnant females used only shallow torpor, but females with litters and juveniles without mothers occasionally were observed in torpor (Juskaitis, 2005). Hibernation in captive wooly dormice (*Dryomys laniger*) lasted from October to April (Kart Gür et al., 2014). Other hibernating dormice include the African dormouse (*Graphiurus murinus*), which also may express torpor throughout the year since torpid animals were observed both in summer (Webb and Skinner, 1996) and winter (Mzilikazi et al., 2012).

In the dipodids (jerboas, jumping mice, birch mice) hibernation from winter to spring has been observed in the Egyptian jerboa, *Jaculus orientalis* (El Ouezzani et al., 2011). As mentioned above, jumping mice (*Zapus princeps*) hibernate in captivity for over 300 days (French, 1985), and just under 300 days (September to early July) at >2,000 m elevation in Utah in the field (Cranford, 1978). Birch mice (*Sicista betulina*) hibernate for 6–8 months (Eisentraut, 1956).

Insectivores

Well-known hibernators in the insectivores (now Lipotyphla) are the hedgehogs. This includes the European hedgehog (*Erinaceus europaeus*), which has been investigated with regard to its hibernation physiology for decades (Kristoffersson and Soivio, 1964; Warnecke, 2017). Danish *E. europaeus*, kept in large outdoor pens, remained within their hibernacula continuously for up to 6 months and the hibernation season lasted from about October to April (Walhovd, 1979) the common seasonal pattern (**Figure 2**). Algerian hedgehogs, *Atelerix algericus*, held individually in a room with open windows near the Mediterranean Sea, commenced the hibernation season with short bouts of torpor in November, expressed long TBDs of 6–7 days in January/February, and ended the torpor season again with short bouts in March (Mouhoub-Sayah et al., 2008). The southern African hedgehog (*Atelerix frontalis*), held under semi-natural conditions in the Karoo, South Africa, hibernated from early May (Autumn) to late July (Winter) and this seasonal pattern is rather rare in the now available data (**Figure 2**). The minimum regulated T_b of *A. frontalis* was 1°C and TBD lasted for up to ∼5 days (Hallam and Mzilikazi, 2011).

Bats

After the rodents (>2,000 species), bats are the second largest mammalian order with around 1,300 species and many, likely the vast majority, can use some form of torpor (Lyman, 1970; Stawski et al., 2014). To my knowledge data on hibernation in bats are currently restricted to the largely insectivorous "microbats". There are no published data on multiday torpor in the largely frugivorous "megabats," however, it is rumoured that some may be able to do it. Although many insectivorous bats are known to hibernate, detailed information on the hibernation season based on transmitter data is not as overabundant as in rodents. To some extent this is related to their small size, which creates technical limitations for tagging them with recording equipment. Transmitters or loggers must be small and therefore will not last for many months as is the case for larger devices used on medium to large hibernators. For these reasons, some bats were captured in winter (e.g., Arlettaz et al., 2000; Hope and Jones, 2012; Jonasson and Willis, 2012) so the exact beginning of the hibernation season remains unknown. Moreover, insectivorous bats are not easily kept in captivity for prolonged periods simply because of their food. However, some microbats are known to show extremely long TBDs in winter lasting up to 45 days as reliably measured by transmitters (Jonasson and Willis, 2012). Another complication with regard to the seasonality of torpor in bats is the lack of a clear distinction between the hibernation season and reproductive season as many bats use torpor during reproduction (Stawski et al., 2014). Bats hibernate under a vast variety of conditions (Webb et al., 1996; Turbill and Geiser, 2008; Meierhofer et al., 2019) and patterns of torpor differ widely among species and habitats. Bats have been reported to have very low minimum regulated T_bs during torpor, often around 0°C or slightly below in cold regions, but even in tropical and subtropical areas minimum regulated T_bs between 4 and 8°C have been measured (Geiser and Stawski, 2011; McKechnie and Mzilikazi, 2011).

It was already recognized over 170 years ago that small insectivorous bats or microbats display multiday torpor in winter and brief bouts of torpor in summer (Hall, 1832). In the pre-hibernation season long-eared bats (*Plecotus auritus*) selected low T_a of ∼10°C, in comparison to summer when they preferred thermo-neutral conditions, and entered torpor for ∼14 h/day as an energy sparing mechanism to enhance fat stores (Speakman and Rowland, 1999). Greater horseshoe bats (*Rhinolophus ferrumequinum*), roosting in caves in southern England, hibernated from mid-October to late May, the most common hibernation season (**Figure 2**), when torpor bouts ranged from ∼1.5 to 12 days (Park et al., 2000). Free-ranging Canadian long-eared myotis (*Myotis evotis*), in addition to hibernation in winter, use torpor on every day between May and August and even when reproductively active (Nagorsen and Brigham, 1993; Chruszcz and Barclay, 2002). Little brown bats (*Myotis lucifugus*) hibernate from about October/November to March/April and express torpor also in summer including during pregnancy and lactation with T_{skin} falling below 10°C (Jonasson and Willis, 2012; Johnson et al., 2019). Daubenton's bat (*Myotis daubentoni*) hibernate (Ransome, 1990), and in late summer in central Germany reproductive females enter torpor mainly during post-lactation, whereas males use torpor frequently even during reproductive period in early summer (Dietz and Kalko, 2006). Pregnant female hoary bats (*Lasiurus cinereus*) entered prolonged torpor with T_{skin} as low as 5.5°C in southern Canada during inclement weather in late spring/early summer. This not only conserved energy, but delayed parturition during the cold spell and bats rewarmed when T_a increased to give birth under more favorable conditions for neonatal survival (Willis et al., 2006).

In warmer regions, Hodgson's bats (*Myotis formosus*) hibernated in abandoned mines in southern Korea from October to June and interestingly maintained $T_{skin} > 11°C$ because of the high T_a (Kim et al., 2013). The rather large (∼60 g) Formosan leaf-nosed bat (*Hipposideros terasensis*) hibernated in abandoned tunnels in Central Taiwan from late December to early March (Liu and Karasov, 2011). Despite rather high T_{skin} of >20°C, TBD was up to 19 days, but periodic rewarming was observed. Subtropical fishing bats (*Myotis vivesi*) hibernated on desert islands in the Gulf of California and expressed torpor in summer when T_as were extremely hot (Salinas-Ramos et al., 2014). Mouse-tailed bats (*Rhinopoma microphillum* and *R. cystops*) fatten in August on winged ants, hibernate with T_{skin} of ∼23°C and partial arousals from late October for 5 months to early spring in geothermally heated caves in cliffs at the Sea of Galilee at T_a ∼20°C (Levin et al., 2015).

Most bats from cold-temperate regions hibernate in thermally stable hibernacula like caves, mines or cellars. However, some bats hibernate in trees. Long-eared bats, *Nyctophilus geoffroyi* and *N. gouldi*, hibernate during winter in trees under exfoliating bark facing the sun (2/3 of observations) or shallow tree cavities (1/3 of observations) in a cool-temperate area in south-eastern Australia (Turbill and Geiser, 2008). Despite partial passive rewarming each day, torpor bouts in mid-winter lasted for 5 days on average with a maximum of 15 days. Arousals were often brief <3 h, but on warm nights it appears that bats were foraging and feeding, explaining how they survive the winter without obvious pre-hibernation fattening. In a tropical area in winter when T_a ranged from 16.5 to 34.0°C, *N. geoffoyi* expressed short bouts of torpor on every day (Geiser et al., 2011). In a cool-temperate area in summer *N. geoffoyi* used short bouts of torpor on warm days on every day and on cool days TBDs up to 2 days were observed (Turbill et al., 2003). During the reproductive period in spring *N. geoffroyi* still used torpor when measured overnight in captivity at T_a 15°C (Turbill and Geiser, 2006). All males, pregnant females and lactating females entered torpor under these rather mild thermal condition, and during torpor, the minimum T_b was only ~0.5°C above T_a, and the minimum TMR was low and similar to that predicted for deep hibernators (Ruf and Geiser, 2015). As variables of torpor in *N. geoffoyi* and also *N. gouldi* did not differ among reproductive groups, it appears that some of the observed differences in torpor patterns in reproductive bats in the wild may be behavioral or ecological rather than physiological (Turbill and Geiser, 2006). For tree-roosting hibernating bats restricted to the tropics and subtropics, such as the northern long-eared bat *N. bifax*, torpor in a subtropical population was observed on 100% of days in winter and 85% of days in summer, TBD lasted for up to 5 days in winter and <1 day in summer, and body mass was indistinguishable between seasons (Stawski and Geiser, 2010b). Moreover, subtropical *N. bifax* expressed more torpor in summer when they were fat than lean suggesting that when they can energetically afford it, they use torpor to minimize foraging requirements and thus exposure to predators (Stawski and Geiser, 2010a).

As some bats enter torpor both in summer and winter and even during reproduction, the question arises whether there is a true seasonal change in physiology. As stated above, the minimum TMR of reproductive *Nyctophilus* bats was as predicted for deep hibernators of the same BM, and only about 10% of that in daily heterotherms on average (Ruf and Geiser, 2015). Similarly, non-reproductive *Nyctophilus* bats, from temperate, subtropical and tropical habitats, did not significantly change TMR with season, their TMR was as predicted for hibernators and was reached within ~4 h when bats were thermo-conforming during torpor entry, and cardiac electrophysiology and heart rates of torpid bats also remained unchanged across summer, autumn and winter (Geiser and Brigham, 2000; Stawski and Geiser, 2011; Currie, 2015, 2018). As TBD is strongly temperature-dependent, and torpor bouts above the minimum regulated T_b are reversely related to T_a (Twente and Twente, 1965; Geiser and Kenagy, 1988), the short and shallow torpor bouts expressed by many microbats at high T_a in summer seem to reflect mainly ambient thermal conditions, rather than a change in physiology. Moreover, long bouts of torpor are expressed in summer in bats and also pygmy-possums (see below) when they are exposed to low T_a. Thus, from a thermal energetics point of view, the brief and shallow torpor bouts in summer seem to be short bouts of hibernation, rather than daily torpor as expressed by daily heterotherms, which have much higher TMRs (Geiser and Brigham, 2000; Stawski and Geiser, 2011) and the same is the case for some marsupial pygmy-possums (see below). These observations suggests that at least in some hibernating bats, there is little or no seasonal change in the physiology of torpor, and do not provide support for the view of strong seasonal phenotypes in hibernators in general.

Primates

Seasonal hibernation has been observed in several species of dwarf lemurs of Madagascar during the cool dry winter (Dausmann, 2014; Dausmann and Warnecke, 2016). The best-studied species is the fat-tailed dwarf lemur (*Cheirogaleus medius*). Although it hibernates from autumn to spring, its pattern of hibernation is rather unusual. Individuals that hibernate in poorly insulated tree hollows, show strong daily fluctuations of T_b with T_a and do not show periodic endogenous arousals. In contrast, *C. medius* that hibernate in well-insulated tree hollows show periodic arousals about once/week (Dausmann and Warnecke, 2016), similar to *C. crossleyi*, hibernating underground (Blanco et al., 2013). However, primate hibernation is not limited to Madagascar. Pygmy slow loris (*Nycticebus pygmaeus*) hibernate from winter to spring in tropical Vietnam (Ruf et al., 2015) albeit their TBDs are brief (up to ~2.5 days) and the normothermic periods between torpor bouts may last for days.

Afrotheria

Heterothermic afrotherians include the tenrecs and golden moles (Tenrecoidea), the elephant shrews (Macroscelidea) and the aardvark (Tubulidentata), which enters shallow torpor during drought (McKechnie and Mzilikazi, 2011; Weyer et al., 2017). Multiday torpor has been observed in the Tenrecoidea and TBDs up to ~2 days in the Macroscelidea. Arguably one of the most unusual patterns of hibernation known is that of the tenrec, *Tenrec ecaudatus*, in subtropical Madagascar. Tenrecs hibernated underground without periodic arousals for up to 9 months, including the summer, with T_bs>22°C and tracking T_{soil} (Lovegrove et al., 2014). Perhaps this species also belongs to the species that can hibernate for one year, but since hibernation was disturbed, this remains to be determined. For the golden mole (*Amblysomus hottentottus longiceps*) in the Drakensberg Mountains of South Africa, data are available only on a single individual and these are very different from the tenrecs. In spring the mole expressed multiday torpor bouts of ~5 days with T_b as low as 8.6°C, interrupted by multiday normothermic periods (Scantlebury et al., 2008). Elephant shrews are often considered to be daily heterotherms, however, captive rock elephant shrews (*Elephantulus edwardii*) remained torpid for up to 44 h with minimum T_b of 9.2°C (Geiser and Mzilikazi, 2011). In the wild, the temporal patterns of torpor in elephant shrews suggest mainly, but not exclusively, daily arousals (Mzilikazi and Lovegrove, 2004) and this is covered below.

Xenarthra

The pygmy armadillo, *Zaedius pichiy*, hibernates from autumn to winter, with torpor bouts lasting for up to 4.5 days (Superina and Boily, 2007). After the hibernation season, pichis continued to show large daily variation in T_b until spring.

Bears and Other Carnivores

Black bears (*Ursus americanus*) maintained under outdoor conditions in Alaska hibernated from November/December to April (Toien et al., 2011). Unlike in other hibernators T_b in bears fell only to about 30°C, but MR was substantially reduced beyond what is expected from temperature-effects demonstrating a strong metabolic inhibition, but from a rather low normothermic MR because of their large size (Toien et al., 2011). Free-ranging brown bears (*U. arctos*) in Sweden reduced activity, T_b and heart rate weeks before they began denning (Evans et al., 2016). Bears entered dens around October/November when T_a was ∼0°C and snow fell and finished denning in early April. During hibernation T_b fell from ∼38 to 33°C and heart rate from ∼70 to 15 beats/per minute. Bears do not eat, drink or defecate during hibernation, but females may give birth and suckle young. Other carnivores that can show multiday torpor bouts occasionally are European badgers (*Meles meles*), but available information suggests that torpor is restricted to winter (Fowler and Racey, 1988).

Monotremes

The only known hibernator in the egg-laying mammals is the short-beaked echidna (*Tachyglossus aculeatus*). It is distributed all over Australia and southern Papua and its seasonal expression of torpor differs accordingly (Nicol and Andersen, 1996). In Tasmania and at high elevations in Kosciuszko NP in south-eastern Australia, the hibernation season lasts for up to 10 months from February/March/April to October/November (i.e., austral summer to spring, **Table 1**; Grigg et al., 1989; Nicol and Andersen, 2007), it is somewhat shorter ∼5 months in the northern Tablelands of NSW (Falkenstein et al., 2001), whereas in Western Australia and Kangaroo island it lasts for 1–3 months (Nicol and Andersen, 1996). Even in the hot and dry climate of south-western Queensland they show torpor bouts of up to 9 days in winter and up to 1 day in summer (Brice et al., 2002). Although in non-reproducing individuals the hibernation season may continue into late spring, reproductively active individuals terminate hibernation in mid-winter (Nicol and Andersen, 2007) and males, which maintain large testes during hibernation, may mate with hibernating females, or with females that aroused from torpor, and, after mating, females expressed multiday, deep torpor (Morrow and Nicol, 2009).

Marsupials

The Monito-del-Monte (*Dromiciops australis*) a south-American opossum (Didelphidae) hibernates (Bozinovic et al., 2004) apparently mainly in winter. More information on seasonal torpor expression is available for the Australian pygmy-possums (Burramyidae). The mountain pygmy-possum (*Burramys parvus*) is an endangered species limited to high altitudes of the Australian Alps (Broome et al., 2012). It hibernates under snow-covered boulder fields from April/May till September or October (austral autumn to spring, **Table 1**) depending on the disappearance of snow cover, and males typically terminate hibernation earlier likely to prepare for reproduction (Körtner and Geiser, 1998). In captivity *B. parvus* reduce activity to enhance fattening during the pre-hibernations season and can almost double their body mass during that time (Körtner and Geiser, 1995). Other pygmy-possums are less seasonal in their expression of torpor. In free-ranging eastern pygmy-possums (*Cercartetus nanus*) in a warm-temperate habitat north of Sydney, multiday torpor of up to 20 days was expressed in winter and short bouts of torpor in summer (Turner et al., 2012a). Observational data suggest that torpor in *C. nanus* occurs in all seasons including early summer, but not in late summer to mid-winter, when banksia trees (*Banksia integrifolia*), a major source of nectar, are flowering and animals reproduce (Bladon et al., 2002). As stated above, captive *C. nanus* can hibernate for up to 1 year (Geiser, 2007) and can do so despite expression of short bouts of torpor lasting less than 1 day at the beginning of the hibernation season likely because even during these brief bouts it can reduce TMR to extremely low values of hibernators (Song et al., 1997) like the *Nyctophilus* bats (see above). These data provide further support for the view that these brief torpor bouts are functionally short bouts of hibernation. Western pygmy-possums (*C. concinnus*) in a Mediterranean climate hibernated in winter expressing both brief and multiday torpor bouts of up to 8 days (Turner et al., 2012b). This species was not examined in summer in the field, but captive *C. concinnus* as well as little pygmy-possums (*C. lepidus*) expressed spontaneous torpor throughout the year, and, when held at a constant T_a of 20°C and natural photoperiod, TBD was affected by photoperiod in *C. nanus* and *C. concinnus* (Turner and Geiser, 2017).

Birds

The only known avian hibernator is the American poorwill (*Phalaenoptilus nuttallii*). Poorwills breed in the western United States and southern Canada and migrate to the southern United States and Mexico in winter where they hibernate (Brigham, 1992; Woods et al., 2019). Data on free-ranging birds from Arizona show that they entered torpor frequently in winter, often at the base of *Opuntia* cacti, but on sunny days T_{skin} fluctuated by >25°C due to passive rewarming by the sun. When birds were artificially shaded they remained inactive for up to 45 days and displayed torpor bouts of 4–7 days, with T_{skin} falling below 5°C (Woods et al., 2019). Qualitatively this pattern of torpor expression is similar to that observed on fat-tailed lemurs (Dausmann, 2014; Dausmann and Warnecke, 2016), although birds and mammals have been phylogenetically separated for ∼300 Million years. In summer in southern Canada, poorwills enter short bouts of torpor (up to 36 h) regularly in spring and autumn but less frequently when incubating and only during inclement weather (Kissner and Brigham, 1993).

DAILY TORPOR

Daily torpor, unlike hibernation, has been more recently discovered (e.g., Bartholomew et al., 1957; MacMillen, 1965; Morhardt and Hudson, 1966; Dawson and Fisher, 1969). Daily torpor is not as obvious as hibernation because animals often forage daily and without physiological measurements it is difficult to ascertain whether an animal is torpid or simply resting or asleep. Overall, data on seasonal torpor expression especially in free-ranging daily heterotherms are rare.

Mammals

Rodents

The seasonality of daily torpor has been investigated in captive Siberian hamsters, *Phodopus sungorus* (Heldmaier and Steinlechner, 1981), and the data suggested that their expression of torpor is strongly seasonal. Spontaneous torpor (food *ad libitum*) in outdoor enclosures was used from October to March (~21% of days in winter) but not in summer (**Figure 3**). Similarly, when held under short photoperiod *P. sungorus* expressed spontaneous torpor, but not under long photoperiod (Geiser et al., 2013). However, torpor could be induced in a summer-acclimated congener, the desert hamster, *P. roborovskii* (Chi et al., 2016). Although it has been suggested that induced torpor and spontaneous torpor in *P. sungorus* differ functionally (Diedrich et al., 2012), some *Phodopus* species obviously have the ability to enter and arouse from torpor even when summer acclimated.

In north-American "mice" (*Peromyscus* spp.), also of the cricetid family, torpor has been investigated in outdoor cages (Lynch et al., 1978; Tannenbaum and Pivorun, 1988). Spontaneous torpor in *P. leucopus* was observed in all seasons (**Figure 3**; Lynch et al., 1978), or in all seasons except summer (Tannenbaum and Pivorun, 1988). The torpor incidence in *P. leucopus* increased substantially to >30% for all seasons by food withdrawal. Similarly, *P. maniculatus* expressed spontaneous torpor in autumn (~4%) and winter (~10%) but not in spring; food withdrawal (**Figure 4**) increased the torpor incidence to 70–78% for all four seasons (Tannenbaum and Pivorun, 1989). Torpor duration and depth for *P. maniculatus* were also similar for all seasons investigated (Tannenbaum and Pivorun, 1988), suggesting little or no seasonal functional change.

Afrotherians

Free-ranging elephant shrews, *Elephantulus myurus*, in KwaZulu Natal, South Africa, expressed torpor throughout the year, but torpor was most pronounced in winter and spring when TBD was ~8–14 h and T_b fell to ~15°C (Mzilikazi and Lovegrove, 2004). However, the maximum TBD of *E. myurus* was 39 h in spring and the minimum T_b 7.5°C in winter, and in captive *E. edwardii* TBDs of almost 2 days and a minimum T_b of 9.2°C, likely a regulated value, were observed. These observations suggest that some elephant shrews may be capable of multiday torpor (see above).

Marsupials

Captive arid zone insectivorous/carnivorous marsupials (*Sminthopsis* spp. and *Dasyuroides byrnei*) held in outdoor enclosures also displayed daily torpor throughout the year (Geiser and Baudinette, 1987). The use of spontaneous (food *ad libitum*) torpor was reduced from 15 to 30% in winter to ~12% in summer (**Figure 3**). Occurrence of induced torpor by withdrawal of food and water showed small seasonal changes in *Sminthopsis* spp. with torpor use increasing from ~75% in summer to 100% of days in winter (**Figure 4**); the latter is the torpor expression for free-ranging individuals in autumn/winter (Warnecke et al., 2008; Körtner and Geiser, 2009). However, *Sminthopsis* spp. did show seasonal changes in thermal energetics with a 2–3°C reduction in the minimum regulated T_b and a 30–40% reduction of the minimum TMR from summer to winter (Geiser and Baudinette, 1987; Geiser, 2007), demonstrating, that although animals can use torpor throughout the year, there is a functional seasonal change in physiology.

The kowari (*Dasycercus blythi/cristicauda*) inhabits sandy and stony deserts in inland Australia. It also changed its expression of torpor with season during the cold season, but unlike in many other species this mainly reflected sex and reproductive state rather than weather or habitat (Körtner et al., 2016). Males expressed torpor after the mating season, females mainly during pregnancy, but not during lactation, and TBD in females was almost twice as long as in males (Körtner et al., 2008, 2016).

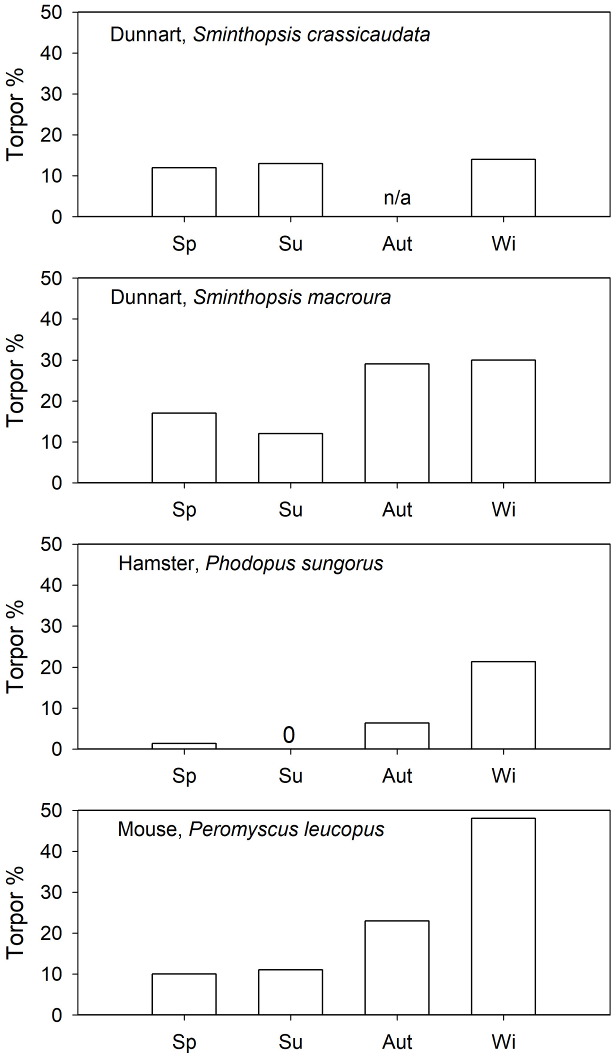

FIGURE 3 | Spontaneous torpor (food *ad libitum*) expression (% of observation days) as a function of season in daily heterotherms. References are provided in the text. 0 = no torpor observed, n/a = data are not available for this season.

Most marsupials of the genus *Antechinus* are forest dwelling. Captive brown antechinus (*A. stuartii*) and yellow-footed antechinus (*A. flavipes*) did not express spontaneous torpor in summer, but occasionally did so in winter. Food withdrawal increased daily torpor expression to about 30–80% from autumn to spring when juveniles are excluded, which did express torpor in summer (Geiser, 1988). In the field, *A. flavipes* expressed torpor in winter, but torpor patterns were strongly affected by reproductive status (Parker et al., 2019), whereas in *A. stuartii*, daily torpor in winter was mainly affected by weather (Hume et al., 2020).

Daily torpor in free-ranging sugar gliders (*Petaurus breviceps*) was observed between autumn and spring and mainly on cold, wet winter days (Körtner and Geiser, 2000b). However, these gliders also expressed daily torpor during a category one cyclone with heavy rainfall in a subtropical area in spring (Nowack et al., 2015).

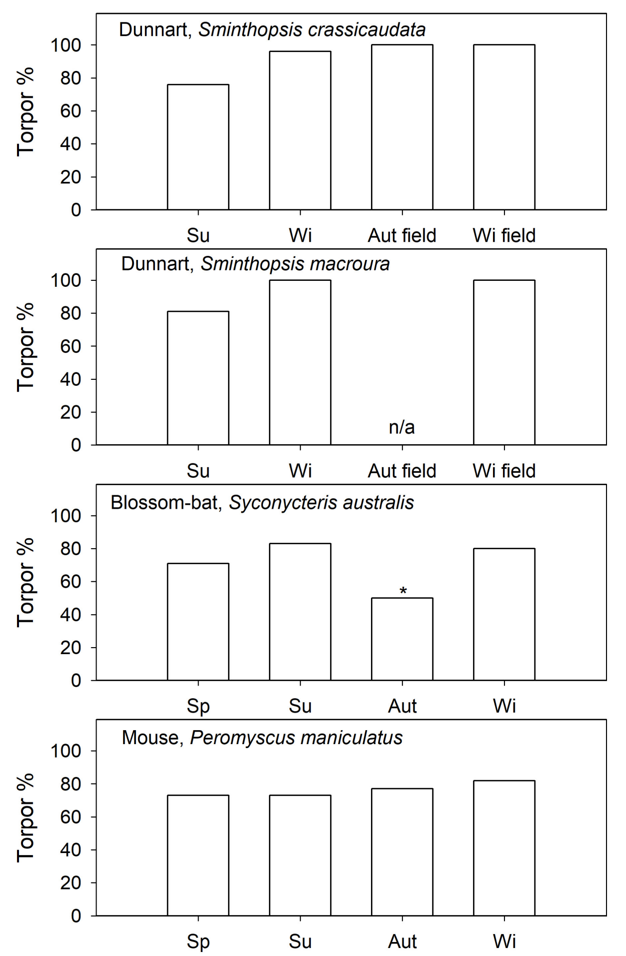

FIGURE 4 | Induced torpor (food restricted) expression (% of observation days) in captive and natural torpor in free-ranging (field) daily heterotherms as a function of season. References are provided in the text. In blossom-bats it is known that they use torpor in autumn, but the % torpor is not available, therefore 50% (*) is assumed. For *S. macroura* field data in autumn are not available.

Birds

Caprimulgiformes

In Australian tawny frogmouths (*Podargus strigoides*), the largest bird known to use torpor, daily torpor was mainly observed on cold winter nights and mornings, and rarely in autumn and spring, summer data are not available. Frogmouths often entered a night torpor bout followed by endogenous rewarming and re-positioning to new roost with camouflaging background to re-enter a morning torpor bout, which was usually terminated by partial passive rewarming in the sun (Körtner et al., 2000, 2001). No torpor occurred during the spring reproductive season between late September and December (Körtner et al., 2001). In owlet-nightjars (*Aegotheles cristatus*) torpor was used at dawn between late autumn and early spring, but not during other times of the year (Brigham et al., 2000). Winter torpor in arid zone owlet-nightjars was much more pronounced during a drought year (Doucette et al., 2012) and birds roosting in trees expressed torpor about twice as often as those in rock crevices (Doucette et al., 2011). Whip-poorwills (*Caprimulgus vociferous*) rarely used torpor in spring or autumn and not in summer (Lane et al., 2004), and it is uncertain what they do during migration to the south.

Apodiformes

Andean Hillstars (*Oreotrochilus estella*) in the Peruvian Andes at ∼4000 m elevation used nocturnal torpor both in winter and summer, but winter torpor was more frequent and longer with extremely low minimum T_bs near 7°C (Carpenter, 1974). Although data on the other seasons are not available it is highly likely that they express torpor throughout the year. Captive Rufous hummingbirds (*Selasphorus rufus*) used torpor from spring to autumn. Rather unusual for daily heterotherms, during pre-migratory fattening in autumn when birds were fat for migration, torpor was most pronounced (Hiebert, 1993). Although winter data are not available because the birds migrate south it is likely that they express torpor throughout the year.

Passeriformes

Alaskan black-capped chickadees (*Poecile atricapilla*) reduced MR during nocturnal torpor to a similar extent in both summer and winter (Sharbaugh, 2001), unlike in the same species and in willow tits (*Parus montanus*) measured at lower latitudes, which expressed shallow torpor only in winter but not in summer (Cooper and Swanson, 1994). In wintering blue tits (*Cyanistes caeruleus*) T_b fluctuations were affected by T_a with higher T_b in experimentally heated tits (Nord et al., 2011), which may explain the differences among populations. Captive passerine sunbirds (*Nectarina famosa*) from South Africa enter nocturnal torpor in summer when exposed to low T_a (Downs and Brown, 2002) suggesting that torpor may also be used at other times of the year. Free-ranging noisy miner (*Manorina melanocephala*) expressed frequent, shallow nocturnal torpor from autumn to early spring (Geiser, 2019) and fairy wrens (*Malurus cyaneus*) in winter, other seasons were not examined (Romano et al., 2019).

"Reversed" Seasonal Torpor Expression

Seasonal changes in the patterns of torpor by nectarivorous blossom-bat *Syconycteris australis* (Megachiroptera) from the subtropical east coast of NSW, Australia, were the opposite of those observed for insectivorous microbats and many other heterothermic mammals, which often display more frequent and more pronounced torpor in winter than in summer. Although induced torpor occurrence was similar in summer and winter (**Figure 4**), average TBD of *S. australis* captured in winter was short (5.5 h) and torpor was shallow with a minimum T_b of ∼23°C, whereas in bats captured in summer torpor was deep (minimum T_b∼19°C) and long at 7.3 h on average (Coburn and Geiser, 1998). The unusual seasonal response seems to be explained by different day length and food availability. In winter, T_a on the subtropical east coast is relatively mild and bats can forage for prolonged periods during long nights and have access to an abundance of flowering plants (Coburn and Geiser, 1998). In summer, nights and thus foraging times are brief and the availability of nectar is substantially reduced (Coburn and Geiser, 1998). Thus, the unusual seasonal pattern of torpor use in *S. australis* appears to be an appropriate physiological adaptation to ecological constraints of their subtropical habitat, but it does suggest a seasonal change in physiology.

For an unrelated heterothermic rodent from the Dead Sea desert region in Israel, similar observations have been made. Spiny mice (*Acomys russatus*) in outdoor enclosures under natural food availability, expressed about twice as many torpor bouts and, on average spent about twice the time in torpor in summer (∼780 min) than in winter (∼370 min) to conserve water (Levy et al., 2011). Even when food was offered *ad libitum*, summer torpor was more frequent and longer than winter torpor.

Seasonal Comparison

Overall, the number of hibernators using torpor (**Figure 5**) differed significantly among the four seasons (Chi Square = 20.5, df = 3, $p < 0.0001$). In contrast, the number of daily heterotherms using torpor did not change with season (Chi Square = 0.97, df = 3, $p = 0.81$). This is further support for the view that there are two major groups of heterotherms suggesting that not only physiological variables, but also the seasonal expression of torpor differs between most hibernators and daily heterotherms.

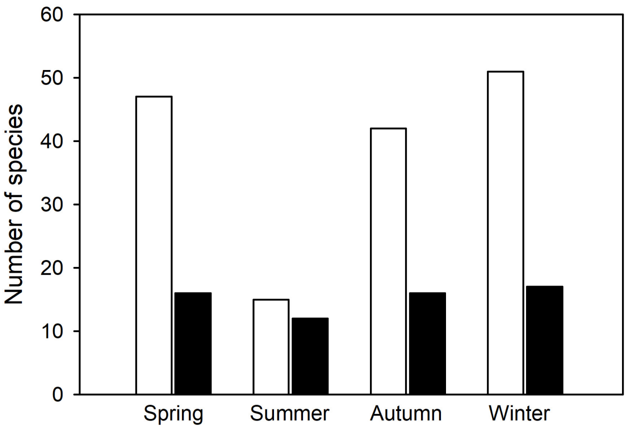

FIGURE 5 | Number of species known to express torpor during different seasons of the year (from **Tables 1**, **2**). In hibernators (white bars), the number of species using torpor changed significantly with season (Chi Square = 20.5, df = 3, $p < 0.0001$), but summer torpor was still used. In daily heterotherms (black bars) there was no change of species number with season (Chi Square = 0.97, df = 3, $p = 0.81$).

What Causes the Seasonal Change in Torpor Expression?

In many species, acclimation or acclimatization to photoperiod or specifically short photoperiod is a strong signal for the preparation for hibernation or the expression of daily torpor. For the hibernators these include dormice, *G. glis* (Morrison, 1964) and European hamsters, *Cricetus cricetus* (Canguilhem et al., 1988). However, more work has been conducted on daily heterotherms such as *Phodopus sungorus* or *Peromyscus maniculatus*, which tend to be highly photoperiodic and respond strongly to exposure to short photoperiod, but low T_a can amplify or accelerate the response in *P. sungorus* (Lynch et al., 1978; Steinlechner et al., 1986; Tannenbaum and Pivorun, 1988; Geiser and Heldmaier, 1995; Hiebert et al., 2003). In *P. sungorus* pelage color, morphology, thermal and reproductive physiology, and tissue fatty acid composition change in response to photoperiod acclimation, but, with regard to seasonal expression of daily torpor, it is especially spontaneous torpor use that shows the strongest response (see above).

In contrast, in many of the sciurid ground squirrels, which are often viewed as "obligate" hibernators, torpor expression is strongly seasonal and highly predictable. Ground squirrels (*Ictidomys tridecemlineatus*), unlike dormice (*G. glis*) held under the same environmental conditions, did not change torpor expression according to photoperiod (Morrison, 1964). Thus in this and other sciurids, the seasonal use of torpor can be more or less independent of photoperiod and to some extent even from T_a and is governed principally by a circannual rhythm (Morrison, 1964; Wang, 1978; Kenagy and Barnes, 1988; Barnes, 1996; Geiser et al., 1990; Michener, 1992; Arnold, 1993; Körtner and Geiser, 2000a; French, 2008; Williams et al., 2017).

In species from low latitudes, such as the subtropical blossom-bat (*Syconycteris australis*) although they may show a strong "reversed" seasonal change in torpor expression when captured in different seasons from the field (see above), photoperiod acclimation in captive individuals did not show a strong effect (Geiser et al., 2005). This suggests that other seasonal signals must be used in the wild. Similarly, the mountain pygmy-possum (*Burramys parvus*), which shows seasonal hibernation in the wild (see above), maintained activity and body mass cycles only within the first winter in captivity (Körtner and Geiser, 1995). Despite maintenance under a mimicked "natural" yearly T_a and photoperiod cycle, this seasonal rhythmicity was lost in the second year in captivity, which again suggest that other seasonal signals must be used in the wild. Therefore, as for

TABLE 2 | The torpor season when daily torpor is used, or is likely to be used, by birds and mammals.

All year

Andean Hillstar, *Oreotrochilus estella*, spring and autumn unknown (Carpenter, 1974)

Rufous hummingbird, *Selasphorus rufus*, migratory, winter unknown (Hiebert, 1993)

Sunbird, *Nectarina famosa*, summer (Downs and Brown, 2002)

Black-capped chickadee, *Poecile atricapilla*, spring and autumn unknown (Sharbaugh, 2001)

Fat-tailed dunnart, *Sminthopsis crassicaudata* (Geiser and Baudinette, 1987; Warnecke et al., 2008)

Stripe-faced dunnart, *Sminthopsis macroura* (Geiser and Baudinette, 1987; Körtner and Geiser, 2009)

Kowari, *Dasyuroides byrnei* (Geiser and Baudinette, 1987)

Blossom-bat, *Syconycteris australis* (Geiser et al., 1996; Coburn and Geiser, 1998)

Elephant shrew, *Elephantulus myurus* (Mzilikazi and Lovegrove, 2004)

White-footed mouse, *Peromyscus leucopus* (Lynch et al., 1978)

Deer mouse, *Peromyscus maniculatus* (Tannenbaum and Pivorun, 1989)

Desert hamster, *Phodopus roborovskii* (Chi et al., 2016; Geiser et al., 2019)

Spiny mouse, *Acomys russatus* (Levy et al., 2011)

Autumn to spring

Tawny frogmouth, *Podargus strigoides* (Körtner et al., 2001)

Owlet-nightjar, *Aegotheles cristatus* (Brigham et al., 2000)

Whip-poorwill, *Caprimulgus vociferous,* migratory, winter unknown (Lane et al., 2004)

Noisy miner, *Manorina melanocephala* (Geiser, 2019)

Brown antechinus, *Antechinus stuartii* (Geiser, 1988; Hume et al., 2020)

Yellow-footed antechinus, *Antechinus flavipes* (Geiser, 1988; Parker et al., 2019)

Sugar glider, *Petaurus breviceps* (Körtner and Geiser, 2000a; Nowack et al., 2015)

Djungarian hamster, *Phodopus sungorus* (Heldmaier and Steinlechner, 1981)

other aspect of seasonal torpor use, the control of its expression differs among species and revealing the responsible cues will require further work.

The Costs and Benefits of Torpor

Avian and mammalian torpor can be highly effective in reducing energy and water use. However, it has been argued that the energy-conserving value of torpor has been overstated (Boyles et al., 2020) and that torpor should be minimized whenever possible because it entails certain risks. Perceived risks include: (i) a physiological dysfunction perhaps due accumulation of waste products at low T_b, (ii) oxidative stress during periodic rewarming, (iii) negative effects on neuronal tissues or memory, (iv) reduced immuno-competence, (v) sleep deprivation, and (vi) increased predation. Many of these risks have been reported as generally applicable (Humphries et al., 2003), although some were based on observations on the eastern chipmunk (*T. striatus*), a non-representative hibernator (see above). Similar concerns of the costs of torpor have been raised by Boyles et al. (2020), but these authors added a nearly complete absence of behavioral responses during torpor (vii) and the increased likeliness of freezing (viii) to the list.

Let us examine these perceived risks in consideration of the seasonal torpor expression and other available information. It is correct that the reasons for periodic arousal from hibernation are still not fully understood and likely involve some malfunction at low T_b, but these can be easily overcome by periodic rewarming and, although this comes at an energetic cost, energy expenditure during hibernation is still only a fraction of that in normothermic individuals. Moreover, in some species hibernating at high T_bs at around 20–25°C such as tenrecs (Lovegrove et al., 2014), hibernation is possible for months without the need to rewarm. Fat-tailed lemurs rely on passive rewarming (Dausmann, 2014) and bats and other species use passive or partially passive rewarming from torpor and thereby minimize energy costs and also the associated oxidative stress (Currie et al., 2015). If oxidative stress does occur in species that do not use passive rewarming, it does not seem to unduly interfere with their wellbeing because heterotherms tend to live longer than homeotherms (Turbill et al., 2011). The memory loss reported for some species, as for example, during hibernation in ground squirrels (Millesi et al., 2001), remains controversial because hibernating bats do not suffer memory loss (Ruczynski and Siemers, 2011). The reduced immuno-competence is a real concern, but often is counteracted by slowed bacterial growth at low T_b. Unfortunately, this is not the case for the new pathogen *Pseudogymnoascus destructans*, a fungus imported 2008/09 from Eurasia, which causes white-nose syndrome in hibernating North American bats, and resulted in catastrophic population declines in many regions (Warnecke et al., 2012). However, more recently surviving bats have developed some immunity (Frick et al., 2017) like their Eurasian counterparts and it appears that survival rates are now improving (Frank et al., 2019). Sleep deprivation during deep torpor (Daan et al., 1991) also can be counteracted by periodic rewarming and again the main costs seems to be energy expenditure, which, as is stated above, is much lower than in normothermic animals despite endogenous rewarming. The perceived increased predation risk during torpor is based on observations of predation of hibernating individuals such as marmots by badgers (Armitage, 2004) or bats by blue tits (Estok et al., 2009). However, even when badgers did find the marmot colony, predation rate was still $<5\%$ (Armitage, 2004) and population studies show that hibernators have much better survival rates during winter hibernation than during the active season in summer (Kawamichi and Kawamichi, 1993; Lebl et al., 2011). Although torpid animals are slower than they are during normothermia, they can move nevertheless, many from around T_b 15°C (Walhovd, 1979; Rojas et al., 2012) and hibernating bats can move at T_b as low as 5–8°C (Choi et al., 1998; Bartonička et al., 2017). With regard to exposure to very low T_a, thermoregulation during torpor does have a negative effect on telomere length (Nowack et al., 2019). However, the likeliness of freezing during hibernation is rather low because of selection of appropriate hibernacula sites, and, even in arctic hibernators that hibernate well below 0°C, endogenous heat production during torpor maintains a large T_b–T_a differential and prevents freezing in most individuals, again at a lower energetic cost than for normothermic individuals (Barnes, 1989; Buck and Barnes, 2000; Richter et al., 2015). Thus, it appears that many of the perceived risks of torpor have been overstated, and the main cost often is a somewhat increased energy expenditure, which is nevertheless much lower than during normothermia. Considering that hibernation is used throughout the year by pygmy-possums, bats and dormice and perhaps other species, and that torpor is used in the presence of food by many species, the view that torpor should be minimized whenever possible is not supported. Instead these and other data on expression of daily torpor for much of the year suggest that the multiple selective advantages of torpor and the diversity of torpor are still not fully appreciated.

AUTHOR CONTRIBUTIONS

The author collected the data and wrote the manuscript.

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MINI REVIEW
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Lifelong Effects of Thermal Challenges During Development in Birds and Mammals

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Before they develop competent endothermy, mammals and birds are sensitive to fluctuating temperature. It follows that early life thermal environment can trigger changes to the ontogeny of thermoregulatory control. At the ecological level, we have incomplete knowledge of how such responses affect temperature tolerance later in life. In some cases, changes to pre- and postnatal temperature prime an organism's capacity to meet a corresponding thermal environment in adulthood. However, in other cases, developmental temperature seems to constrain temperature tolerance later in life. The timing, duration, and severity of a thermal challenge will determine whether its impact is ameliorating or constraining. However, the effects influencing the transition between these states remain poorly understood, particularly in mammals and during the postnatal period. As climate change is predicted to bring more frequent spells of extreme temperature, it is relevant to ask under which circumstances developmental thermal conditions predispose or constrain animals' capacity to deal with temperature variation. Increasingly stochastic weather also implies increasingly decoupled early- and late-life thermal environments. Hence, there is a pressing need to understand better how developmental temperature impacts thermoregulatory responses to matched and mismatched thermal challenges in subsequent life stages. Here, we summarize studies on how the thermal environment before, and shortly after, birth affects the ontogeny of thermoregulation in birds and mammals, and outline how this might carry over to temperature tolerance in adulthood. We also identify key points that need addressing to understand how effects of temperature variation during development may facilitate or constrain thermal adaptation over a lifetime.

Keywords: body temperature, climate change, development, endotherm, heterothermy, phenotypic flexibility, temperature fluctuation, thermal adaptation

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INTRODUCTION

Mammals and birds are endotherms and, as such, control core body temperature (T_b) by means of endogenous heat production across a vast temperature span. However, for the duration of embryonic development, and at least until adequate insulation has been attained, these animals are effectively poikilothermic, i.e., have limited ability to maintain T_b when ambient temperature (T_a) fluctuates (e.g., Pereyra and Morton, 2001; Geiser et al., 2019). Hence, parents buffer changes in T_a

to secure appropriate developmental conditions until offspring have attained endothermy. This is pivotal, because low T_b slows growth rate which may prolong both the embryonic period and time to independence with potential downstream ecological consequences (Remes and Martin, 2002; Cheng and Martin, 2012). Yet, because parents also need to self-feed and, in the case of altricial species, periodically leave the nest to provide for offspring, developing endotherms will be subjected to fluctuating T_a, at least for some periods in some early life stages.

Given the sensitivity to perturbations when regulatory systems form (Burggren and Mueller, 2015; Eyck et al., 2019), the embryonic thermal environment can affect pre- and postnatal phenotypes. When there is substantial and sustained deviation from optimum developmental temperature, offspring may accrue congenital deficiencies (e.g., Lundy, 1969). Such pronounced challenges are arguably rare in nature. It may therefore be more relevant to consider effects of lower-intensity temperature variation, such as during unusually cold or warm breeding seasons, across a reproductive season, and in relation to variation in parents' reproductive investment. This has been studied in some detail in poultry (e.g., Tzschentke and Nichelmann, 1999; Nichelmann and Tzschentke, 2002; Nichelmann, 2004). Broadly speaking, these efforts show that mild, short-duration, thermal stimuli before or shortly after hatching improve chicks' capacity to deal with a corresponding challenge as juveniles and in adolescence. However, this work has unclear ecological relevance, because free-ranging animals are presumably adapted to more variable thermal environments, and face different thermoregulatory and energetic constrains, than poultry (e.g., Tickle et al., 2018; Tickle and Codd, 2019). Thus, work on wild birds indicates that mildly hypothermic incubation reduces, whereas mildly hyperthermic incubation increases, offspring cold tolerance (e.g., DuRant et al., 2012, 2013a,b). In contrast, short- and long-term effects of changes to rearing temperature on thermoregulation are poorly understood. In mammals, it is not known how offspring thermoregulation is affected by prenatal temperature, and data on rearing temperature-effects on thermal physiology in subsequent life stages are scarce.

The lack of information on how developmental temperature affects adult thermoregulation is unfortunate, not the least considering the predicted increase of extreme temperature events (IPCC, 2013) that risks increasingly decoupling juvenile and adult thermal environments. It is conceivable that developmental-temperature-effects on adult thermoregulation can be broadly categorized as ameliorating or constraining (**Figure 1**): (a) if juveniles adapt non-reversibly to their thermal environment, then as adults we expect individuals to perform better in matched, and worse in mismatched, environments ("Environmental matching hypothesis"; **Figure 1A**); (b) if changes to developmental temperature constrain juvenile growth and maturation, we expect that individuals who were thermally challenged when growing up will consistently perform worse than those that developed in "normal" environments as adults ("Silver spoon hypothesis"; **Figure 1B**) (terminology after Monaghan, 2008). Here, we summarize the main findings for how developmental temperature affects the ontogeny of thermoregulation and how this links to adult thermoregulatory performance. We discuss the extent to which this may facilitate or constrain thermal adaptation in adulthood, and finish by addressing particularly pressing matters to investigate in this context.

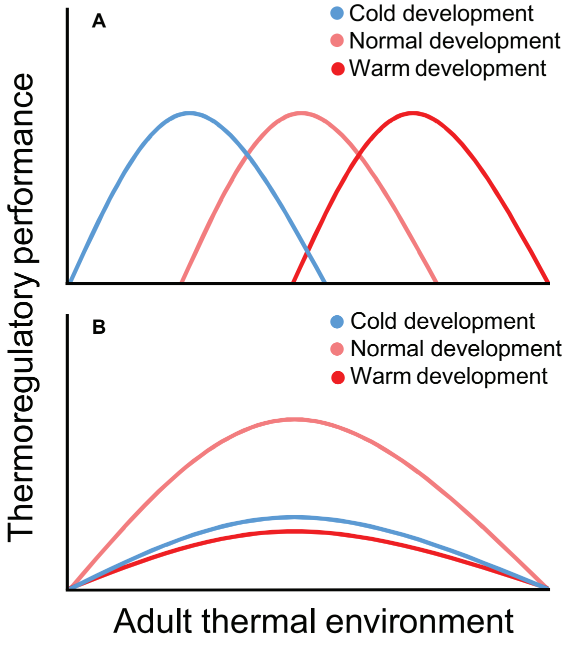

FIGURE 1 | Possible effects of the thermal environment during development on thermoregulatory performance in adulthood. Under the environmental matching hypothesis **(A)**, adult performance is better when the thermal environment matches that in which the individual developed. Under the silver spoon hypothesis **(B)**, both colder-than-normal and warmer-than-normal developmental temperatures act suppressively on the pre- and/or postnatal phenotype, such that individuals that developed in unconstraining thermal regimes (here, "normal") consistently perform better than cold- and warm-reared individuals as adults.

WHEN AND WHY DOES DEVELOPMENTAL TEMPERATURE VARY?

Mammals Before Parturition

Females of many mammals improve embryonic homeothermy by reducing circadian variation in T_b during gestation (e.g., Fewell, 1995; Trethowan et al., 2016; Wharfe et al., 2016; Thiel et al., 2019), and may even suppress febrile responses to protect the embryo from thermal damage (Begg et al., 2007) (but see Laburn et al., 1992). Even some heterotherms, which would normally display large daily or seasonal T_b reduction, are more homeothermic during pregnancy. For example, hibernating bears maintain stable T_b during gestation and only allow T_b to drop after parturition (Hissa, 1997; Tøien et al., 2011; Shimozuru et al., 2013; Friebe et al., 2014). Other heterotherms do use torpor when pregnant. This is typically a direct or preemptive response to energy shortage and is more common in species regularly facing energetic challenges during breeding, such as those reproducing when it is cold and those relying on ephemeral or seasonal/patchy forage (reviewed by Geiser, 1996; McAllan and Geiser, 2014). Females safeguarding energy balance in this manner do so at the expense of prolonged gestation (e.g., Racey, 1973) and possible phenotypic consequences to offspring resulting from a more variable developmental temperature.

Mammals After Parturition

Until thermogenic capacity is sufficient for self-maintenance, mammals experience fluctuating T_b as determined by the amount of maternal brooding and thermal properties of the nest, and by the extent to which post-parturition females use torpor. Some of the fluctuations in nest temperature can be mitigated by huddling, which allows maintained growth rate even during a cold challenge (Gilbert et al., 2007, 2010, 2012). After weaning, young mammals are inevitably exposed to fluctuating T_a in line with habitat properties. Depending on reproductive period, juveniles of the same species might experience warm or cold temperatures during this time.

Birds Before Hatching

Because birds have external development, embryos are more exposed to T_a compared to (non-monotreme) mammals. With some exceptions, such as the megapodes that utilize heat from decomposing material to incubate eggs (Booth and Jones, 2002) and some species that rely on solar incubation (e.g., De Marchi et al., 2008), heat for embryonic development is actively supplied by one or both parents. Some capital breeders, such as common eiders (*Somateria mollissima*), take no or few daily recesses from incubation (e.g., Kristjánsson and Jónsson, 2011). At the other extreme are single-sex intermittent incubators that leave the nest to forage several times per hour (Deeming, 2002). It follows that parental incubation behavior impacts the degree of temperature fluctuation experienced by the embryo. Moreover, incubation temperature is often lower in more strenuous conditions, such as in low T_a or during incubation of larger clutches (reviewed by Nord and Williams, 2015), because the energy costs of incubation constrain parental investment in keeping eggs warm (Williams, 1996; Tinbergen and Williams, 2002; Nord and Williams, 2015). Females of some species mitigate these costs by torpor (Calder and Booser, 1973; Kissner and Brigham, 1993), with inevitable consequences for embryonic temperature.

Birds After Hatching

Once eggs hatch, chicks are brooded by one, or both, parents until thermogenic capacity and insulation are sufficient. Precocial species, that self-feed from hatching onward, are exposed to the elements during this time and will alternate short feeding bouts with being brooded by the parents (e.g., Pedersen and Steen, 1979). Altricial chicks are more strongly affected by T_a, meaning T_b is influenced by the balance between parental provisioning and brooding. However, on account of the increase in thermal mass as chicks grow, the brood as a unit may be functionally homeothermic already a few days after hatching (Węgrzyn, 2013; Andreasson et al., 2016).

RESPONSES TO PRENATAL TEMPERATURE VARIATION

Mammals

We are not aware of any studies that have tested how fluctuating temperatures *in utero* affect the subsequent thermoregulatory performance of juveniles and adults. This clearly needs further investigation (see section "Future Directions" below).

Birds

The effects of embryonic temperature on postnatal thermoregulation have been studied particularly in poultry since temperature fluctuations inside rearing facilities have consequences for welfare and economic return (Naga Raja Kumari and Narendra Nath, 2018). Thermal sensitivity is the greatest when the hypothalamus-thyroid-pituitary-adrenal (HTPA) axis forms (Loyau et al., 2015), in line with the modulatory role of thyroid hormones in avian thermoregulation (Ruuskanen et al., 2019). In the chicken, this commences during the middle third of embryogenesis, when even brief (2–5 h) exposure to hypo- or hyperthermic incubation alters thyroid and glucocorticoid hormone secretion in response to a thermal challenge after hatching, and results in phenotypic changes that improve chicks' capacity to deal with cold or heat at least until market age of ca. 35–50 days (e.g., Yahav et al., 2004; Shinder et al., 2009, 2011; Piestun et al., 2011). However, the effects appear to be different when the challenge is continuous. For example, periodic cooling during the entire incubation period in zebra finches (*Taeniopygia guttata*) increased embryonic metabolic rate, but decreased yolk conversion ratio, such that chicks hatched in poorer condition (Olson et al., 2006, 2008). Similarly, chickens incubated at constant low temperature produced less, not more, heat during acute cold exposure compared to controls (Black and Burggren, 2004). Moreover, Japanese quail (*Coturnix japonica*) chicks incubated in constant or cyclical low temperature were smaller, weighed less, and had elevated metabolic rate (after constant low incubation only) as adults relative to controls (Ben-Ezra and Burness, 2017).

Embryos of wild birds are adapted to the constantly fluctuating temperatures produced by parental behavior (above, and Webb, 1987). Yet, studies directly manipulating egg temperature in free-ranging birds largely corroborate findings in captive models. Accordingly, chronically low incubation temperature lowers body condition and elevates metabolic rate (Hepp et al., 2006; DuRant et al., 2011; Nord and Nilsson, 2011), and reduces the capacity to meet a cold challenge (DuRant et al., 2012, 2013a). None of these studies measured effects on thermoregulation once chicks were independent. Hence, it is unclear if incubation temperature-linked effects on survival in wild birds after fledging (Hepp and Kennamer, 2012; Nord and Nilsson, 2016; see also Berntsen and Bech, 2016) has a thermo-physiological basis.

It is not known if brief exposure to low incubation temperatures, similar to that in many poultry studies, affects offspring thermoregulation. This is unfortunate, because incubating birds sometimes prioritize self-maintenance by ceasing to incubate for several hours (e.g., MacDonald et al., 2013; reviewed by Nord and Williams, 2015). The resultant thermal challenge for embryos may be equivalent to when mammals enter torpor during gestation.

RESPONSES TO POSTNATAL TEMPERATURE VARIATION

Mammals

We are aware of only two studies reporting on how early life thermal conditions affect thermoregulation in adult mammals. In fat-tailed dunnarts (*Sminthopsis crassicaudata*), adults showed more frequent, deeper, torpor associated with significantly greater energy savings when they developed, and were subsequently kept, in cold compared to warm conditions (Riek and Geiser, 2012). In yellow-footed antechinus (*Antechinus flavipes*), rearing in warm conditions from weaning onward caused increased metabolic rate when adult females, but not males, were cold-exposed. After warm-exposure of adults that were reared in the cold, metabolic rate was significantly reduced for both sexes (Stawski and Geiser, 2020). Hence, developing in the warmth seems to reduce flexibility of the metabolic response to changing temperature, at least in males. In line with this, piglets exposed to heat stress during their first 10 days of life showed reduced thermo-tolerance when heat stressed at weaning compared to piglets reared in standard and cold conditions (Johnson et al., 2018).

Developmental temperature also affects morphology. In rats, warm-rearing from parturition increases the size and vascularity of thermolytic effectors (tail, salivary glands) (Demicka and Caputa, 1993a,b). While vascularity is likely amenable to subsequent thermal acclimation (e.g., Demicka and Caputa, 1993a), changes to external morphology (and associated heat transfer consequences) could remain over the animals' lifespan.

Birds

In the chicken, thermal manipulation for 12–24 h during the first week after hatching elicits responses largely analogous to those triggered by the same stimulus during incubation. Accordingly, heat- or cold-acclimation at this age improves control of T_b and survival when chicks are subsequently exposed to acute thermal stress at 6–7 weeks of age (Arjona et al., 1988, 1990; Yahav and Hurwitz, 1996; Shinder et al., 2002), possibly *via* acclimation of evaporative cooling capacity (Marder and Arieli, 1988; Midtgård, 1989). It is not clear if the causation is similar to that in the embryonic period. However, non-thermal challenges to young birds can bring lasting effects on glucocorticoid levels (e.g., Marasco et al., 2013), which suggest that the HTPA axis is still sensitive to developmental perturbations at this time.

In line with studies on mammals, postnatal T_a can affect the size of thermolytic effectors. Japanese quail reared in warm T_a developed smaller bills than birds reared in cold T_a (Burness et al., 2013). As adults, after nearly 3 months in common garden, warm-reared quail had higher bill temperature than cold-reared birds, particularly in low T_a, indicating non-reversible changes to bill vasculature (Burness et al., 2013). It would be interesting to know if heart-weight reduction in warm-reared chickens (Yahav and Hurwitz, 1996), which has obvious links to circulation and thermoregulation, is equally non-reversible.

Only a handful of studies have manipulated rearing temperature in wild birds, with context-specific ameliorating or suppressing effects on growth, depending on the thermal environment where the manipulation was performed (Dawson et al., 2005; Rodríguez and Barba, 2016a,b; Andreasson et al., 2018). Only one of these studies gives some insight into effects on thermoregulation: Andreasson et al. (2018) found that heated chicks maintained stable T_b throughout ontogeny despite T_a approaching 50°C, even at ages where control chicks were poikilothermic. Hence, at least part of the suppressive effects of postnatal T_a might reflect differential allocation of resources from growth to thermoregulation to avoid hypo- or hyperthermia. It is not known if any such changes remain until adulthood. However, the fact that warm T_a improved chick survival in a cold habitat (Dawson et al., 2005) and long-term survival in an intermediate thermal environment (Andreasson et al., 2018), but negatively affected survival in a hot and dry climate (Rodríguez et al., 2016), suggests this is a topic worthy of future investigation. In this context, it is interesting to note that in altricial birds (like those in the studies above), the HTPA axis matures during the first week after hatching (Debonne et al., 2008), which suggests that thermal sensivity may be greater postnatally than in precocical species.

FUTURE DIRECTIONS

There appears to be broad synergies between studies in birds and mammals, despite variation in timing, duration, and severity of thermal stressors. In birds, there is a bias toward studies of production species with unclear ecological relevance, a general lack of information on effects of postnatal temperature on thermoregulation, and poor understanding of when a thermal dose is constraining or ameliorating. Mammals are comparatively understudied in all these regards. Hence, it is clear that more studies are needed to address how developmental temperature affects the ontogeny of thermoregulation and how this, in turn, impacts thermal physiology of adults. Below we outline some directions to further our knowledge of these matters (**Figure 2**):

- *(i) Fill in the blanks:* For example, there are few studies of thermal sensitivity of mammalian embryonic development and its short- and long-term consequences, despite widespread occurrence of heterothermy during pregnancy. In birds, there are no studies of how developmental temperature affects energy-conserving strategies, despite widespread heterothermy in this phylum (McKechnie and Lovegrove, 2002), and it is unknown how chick thermoregulation is affected by egg neglect.
- *(ii) Predisposing or constraining:* Increasingly stochastic climate suggests increased likelihood that an animal will develop during extreme weather, or that it will experience such events sometime during its lifetime. A key challenge is therefore to address if, how, and why, physiological changes that manifest during development affect performance when

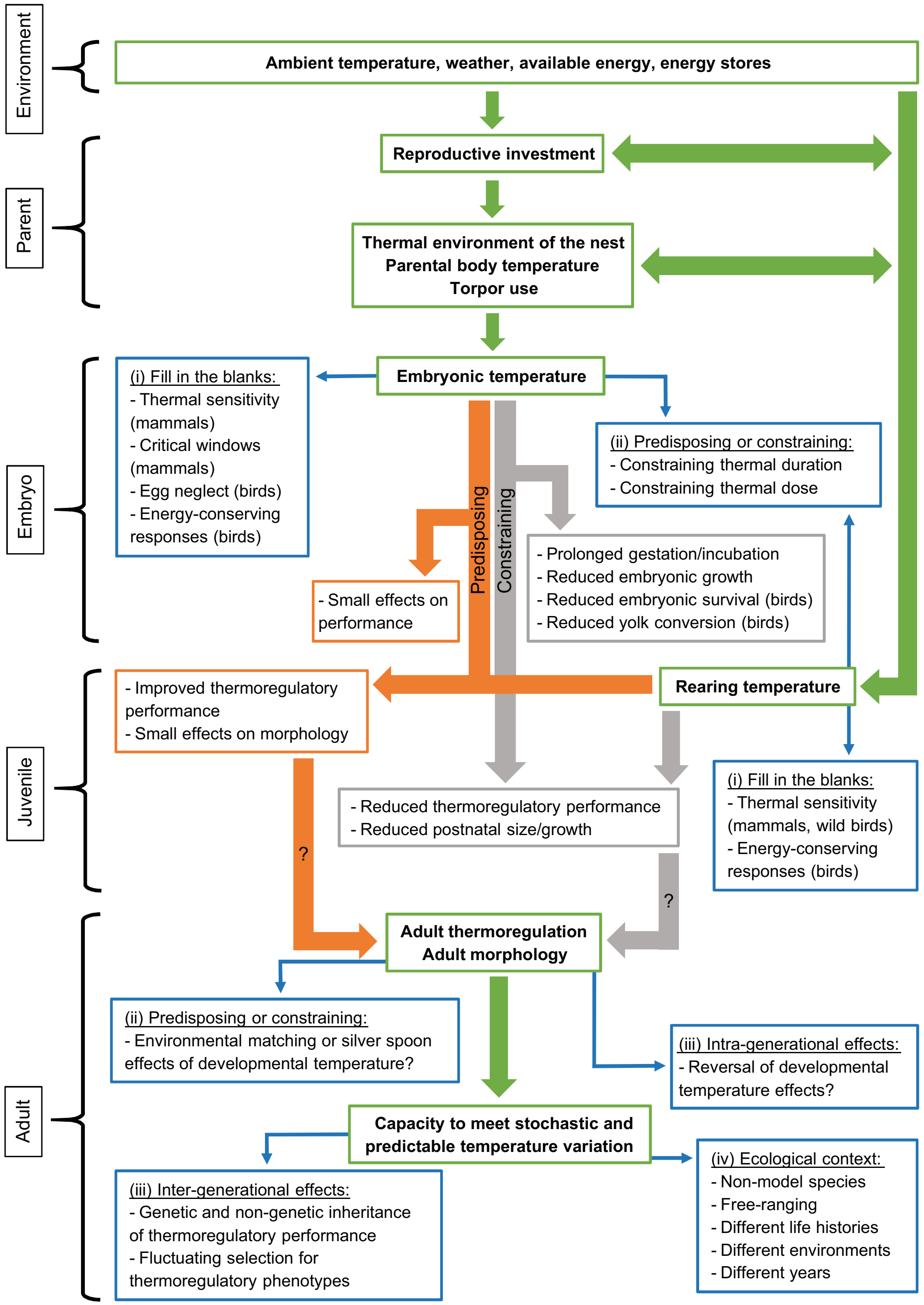

FIGURE 2 | A putative flow path linking breeeding environment, parental investment, developmental and rearing temperatures, and thermal performance in adulthood. Main connections outlining how environmental and intrisic conditions experienced by parents during the breeding season can affect reproductive investment and resultant embryonic and postnatal thermal environments are shown using green arrows, with relevant descriptors in bold font within the green boxes. It is assumed that early life temperature can be either predisposing or constraining for subsequent thermoregulatory performance. These developmental trajectories are shown by orange and gray arrows, respectively, with relevant effects summarized within the orange and gray boxes. Paths where data are scarce, or even lacking, are traced using thin blue arrows, with key knowledge gaps listed within the blue boxes. For simplicity, these are referred to by the Roman numerals in the section "Future Directions."

the juvenile and adult environments are mismatched. While subtle, short-duration, variation in developmental temperature can improve thermoregulatory performance in the same environment later in life, there are switch points where early-life temperature constrains subsequent temperature tolerance (e.g., Costantini et al., 2012). We need to understand better when a thermal dose transitions from predisposing to constraining, the phenotypic changes involved, and their epigenetic underpinnings (e.g., Vinoth et al., 2018; Wang et al., 2019). In this context, there is also a need for studies across life histories. For example, is environmental matching as relevant in a trans-continental migrant compared to a year-round resident (cf. Yin et al., 2019)?

- *(iii) Intra- and intergenerational effects:* There is a need to increase our understanding of the extent to which the thermo-physiological effects of developmental temperature remain over a lifetime, especially in wild models and mammals. To understand the evolution of responses, studies should address if traits that are differentially expressed in different developmental temperatures are heritable (cf. Rønning et al., 2007; Versteegh et al., 2008; Nilsson et al., 2009).
- *(iv) Broader ecological context:* Studies of physiological effects have used captive models, but fitness costs have been documented in the wild with little information on physiological mediators. We need to apply theory derived from captive models to wild animals that live under fluctuating T_a in a range of habitats, to better understand the eco-evolutionary dynamics of developmental thermal sensitivity.

CONCLUSION

It is clear that mammals and birds are sensitive to fluctuating developmental temperature in broadly similar ways, and that changes brought about by the early thermal environment sometimes may permanently modify the phenotype. To this end, effects of temperature resemble those of other environmental factors during development (Costantini et al., 2010). Some studies, particularly in poultry, adhere to the environmental matching hypothesis (**Figure 1A**), showing that thermal acclimation in early life (*via* well-timed, brief, thermal manipulation) improves the capacity to meet matched stimuli in adulthood. However, wild and captive studies where the thermal challenge has been continuous (and the ecological relevance greater) adheres more closely to the silver spoon hypothesis (**Figure 1B**). That is, sustained deviation from the thermal environment to which the population is adapted seems to constrain phenotypic quality. However, we caution against general conclusions in this regard, because many key studies are yet to be performed (above and **Figure 2**), particularly in wild systems. Furthering our knowledge on how early life thermal conditions shape thermoregulatory phenotypes is more pressing now than ever when climate change increasingly exposes animals to extreme weather (IPCC, 2013) with potentially severe consequences (McKechnie and Wolf, 2010; Conradie et al., 2019; Riddell et al., 2019). Proper understanding of the ontogeny and acclimatization capacity of, and selection for, temperature tolerance is, thus, key to predicting how individuals and populations will respond to such challenges (cf. Stillman, 2003, 2019; Burggren, 2018). We hope that this review will inspire others to collect the data needed for better understanding of these effects.

AUTHOR CONTRIBUTIONS

AN and SG together developed the concepts of this mini-review, drafted its outline, and revised the manuscript. AN wrote the full version and produced the figures.

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Ambient Temperature Cycles Affect Daily Torpor and Hibernation Patterns in Malagasy Tenrecs

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Hibernation and daily torpor (heterothermy) allow endotherms to cope with demanding environmental conditions. The depth and duration of torpor bouts vary considerably between tropical and temperate climates, and tropical hibernators manage to cope with a wider spectrum of ambient temperature (T_a) regimes during heterothermy. As cycles in T_a can have profound effects on activity and torpor patterns as well as energy expenditure, we examined how these characteristics are affected by daily fluctuating versus constant T_a in a tropical hibernator, the lesser hedgehog tenrec (*Echinops telfairi*). Throughout the study, regardless of season, the tenrecs became torpid every day. In summer, *E. telfairi* used daily fluctuations in T_a to passively rewarm from daily torpor, which led to synchrony in the activity phases and torpor bouts between individuals and generally decreased energy expenditure. In contrast, animals housed at constant T_a showed considerable variation in timing and they had to invest more energy through endogenous heat production. During the hibernation season (winter) *E. telfairi* hibernated for several months in constant, as well as in fluctuating T_a and, as in summer, under fluctuating T_a arousals were much more uniform and showed less variation in timing compared to constant temperature regimes. The timing of torpor is not only important for its effective use, but synchronization of activity patterns could also be essential for social interactions, and successful foraging bouts. Our results highlight that T_a cycles can be an effective *zeitgeber* for activity and thermoregulatory rhythms throughout the year and that consideration should be given to the choice of temperature regime when studying heterothermy under laboratory conditions.

Keywords: hibernation, torpor, body temperature, *zeitgeber*, tropics, *Echinops telfairi*

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INTRODUCTION

Limited food and water supply and unfavorable climatic conditions often require mammals to find means to reduce their dependency on energy and water availability. This holds particularly true for small animals in harsh climates as their small surface area to volume ratios lead to greater heat transfer and water loss. Hibernation, prolonged and daily torpor (combined here as heterothermy) are physiological strategies that allow endotherms to cope with such demanding conditions (Lyman et al., 1982). These physiological states are characterized by an active depression of metabolic rate (MR) and a change in set point of body temperature (T_b, Geiser, 2004; Heldmaier et al., 2004), providing high savings in energy and water and a lowered T_b that can be close to ambient temperature (T_a) during deep torpor (Ruf and Geiser, 2015). Daily torpor is limited to short bouts of less than 24 h, prolonged torpor bouts last for a few days, whereas hibernation consists of a series of approximately 1–2 week-long torpor bouts interspersed by active arousals, usually totaling several months (Geiser, 2004; Heldmaier et al., 2004; Nowack et al., 2020). Although heterothermy is best known, and probably more common in arctic and temperate climates, it also occurs in the tropics (Cossins and Barnes, 1996; Ruf and Geiser, 2015; Nowack et al., 2020). With increasing numbers of ecophysiological field studies there is more and more evidence of heterothermy in tropical mammals: e.g., bats (Stawski and Geiser, 2011; Reher et al., 2018), cheirogaleid lemurs (Dausmann, 2014), the bushbaby *Galago moholi* (Nowack et al., 2010), loris (Ruf et al., 2015; Streicher et al., 2017), tenrecs (Lovegrove and Génin, 2008; Oelkrug et al., 2013; Levesque et al., 2014), birds (McKechnie and Mzilikazi, 2011), echidnas, and marsupials (Grigg and Beard, 2000; Geiser and Körtner, 2010; Körtner et al., 2010). In fact, there are more mammalian orders with heterotherms than without, and it is likely that the capacity for heterothermy is the ancestral state in mammals (Grigg et al., 2004; Lovegrove, 2012a,b).

In contrast to arctic or temperate regions, the ultimate triggers of heterothermy in the tropics might not be as straight forward, and are more multifaceted than low temperature and low availability of food (see Geiser and Brigham, 2012; Nowack et al., 2017, 2020). The costs of endothermy might be less pronounced in many parts of the tropics, however, food and water can be scarce during all or some periods of the year. Thus, the water saving potential of hibernation and daily torpor becomes more important in the tropics (Macmillen, 1965; Cryan and Wolf, 2003; Schmid and Speakman, 2009). Reductions in MR, T_b, food requirements and activity are accompanied by reductions in evaporative, fecal and urinary water loss, leading to substantial water savings (Cooper et al., 2005; Withers et al., 2012). Similarly, the time course and pattern of T_b during hibernation varies considerably between tropical and temperate climates. Arctic and temperate hibernators encounter very low T_a and subsequently exhibit very low T_b during hibernation, sometimes even below the freezing point (Barnes, 1989; Pretzlaff and Dausmann, 2012). Additionally, hibernacula of arctic and temperate hibernators are generally well insulated and temperature fluctuations are small within the hibernaculum (Arnold et al., 1991; Buck and Barnes, 1999). Tropical hibernators, on the other hand, use hibernacula with very variable degrees of insulation capacities, e.g., hollows in trees of varying heights and thicknesses, or underground sites at varying depths (Dausmann et al., 2004; Körtner et al., 2010; Blanco et al., 2013; Levesque et al., 2014; Lovegrove et al., 2014a). Therefore, depending on the choice of hibernaculum or resting site, i.e., well versus poorly insulated, tropical hibernators manage to cope with a wide spectrum of temperature regimes during hibernation: from constant to highly fluctuating temperatures, with elevated temperatures possibly enhanced by tropical solar radiation during the day. As T_b usually approximates T_a during hibernation, this flexibility is also reflected in T_b (Dausmann et al., 2004; Kobbe and Dausmann, 2009; Canale et al., 2012; Levesque et al., 2014; Reher et al., 2018). For example, the range of daily T_a fluctuations affects the hibernation pattern in the lemur *Cheirogaleus medius* (Dausmann et al., 2005) and many heterotherms use the daily T_a fluctuations to assist warming up from daily and prolonged torpor or hibernation bouts (Ortmann et al., 1997; Schmid, 2000; Mzilikazi et al., 2002; Turbill and Geiser, 2008; Warnecke et al., 2008; Kobbe and Dausmann, 2009; Warnecke and Geiser, 2010; Thompson et al., 2015). However, T_a cycles not only help rewarming, they also act as a *zeitgeber*, influencing activity patterns (Pohl, 1998; Vivanco et al., 2010).

We therefore sought to characterize the effects of differing T_a patterns on the thermophysiology of a tropical hibernator, the lesser hedgehog tenrec (*Echinops telfairi*). We aimed to evaluate how the choice of hibernaculum (i.e., insulation capacity) influences hibernation parameters in the wild by examining how daily fluctuating T_a versus constant T_a affects patterns of daily torpor and hibernation and energy expenditure. As the previous measures of cost of hibernation under constant T_a conditions may have overestimated the total frequency and cost of rewarming in tropical hibernators, we measured metabolic rate to test if T_a fluctuations are used to assist with warming during arousals. Finally, by simulating a range of summer and winter temperatures, we aimed to analyse how hibernation patterns and energy expenditure are affected by variable and changing temperatures during hibernation.

MATERIALS AND METHODS

Study Species

Echinops telfairi (Martin, 1838) is a small (135 g) nocturnal insectivorous member of the family Tenrecidae and endemic to Madagascar (Eisenberg and Gould, 1969). It uses daily torpor during the austral summer, and hibernates during the winter. It has one of the lowest reported euthermic T_b of any eutherian mammal and is highly thermally labile (Scholl, 1974; Clarke and Rothery, 2008; Lovegrove and Génin, 2008). In Madagascar, *E. telfairi* rests and hibernates in tree hollows, dead trees or under leaf litter (Eisenberg and Gould, 1969; Soarimalala and Goodman, 2011). Neither offer a particularly well-insulated resting site and it can thus be assumed that they experience fluctuating T_a year-round.

Eighteen female and nine male adult, laboratory-bred lesser Malagasy hedgehog-tenrecs (*E. telfairi*; 3–5 years old during the experiments) were used for the experiments over a two year time period. The animals were acquired from the Ludwig-Maximilians-University Munich, where they had been bred for over 30 years and fully acclimated to northern hemisphere seasonal rhythms (e.g., Künzle, 1998). All animals were earmarked or marked with an injectable micro transponder (ID-100, Trovan, Usling GmbH, Weilerswist, Germany), to unambiguously identify individuals.

Experimental Setup

The animals were kept in separate cages (35 × 21 × 35 cm, L × W × H) in a climate chamber (Type TCR + 2, Weiss Technik, Reiskirchen, Germany) during the experiments and weighed regularly. The cages were equipped with wooden nest boxes (14 × 20 × 14 cm), wood chips, a hamster wheel and other environmental enrichment. Food (mealworms, cockroaches, wet canned cat food, dry dog food, dry hedgehog food, boiled egg and fresh fruit) and water were provided *ad libitum*. To test for the effects of T_a on torpor patterns the animals were exposed to five different temperature treatments, two during the animals' summer and three in their winter. Temperatures were chosen to match actual climatic conditions of *E. telfairi* in their natural resting sites (Jury, 2003; Dausmann and Blanco, 2016). During summer, day length (simulated by ambient lighting) and humidity were adjusted to 13 h and 70%, respectively. T_a was either held constant at 24°C ($S_{const24}$) or fluctuating between 19°C during the dark phase and 28°C during the light phase ($S_{fluc19-28}$). In winter day length was reduced to 11 h and humidity to 40%, constant T_a was set at 18 or 12°C ($W_{const18}$ and $W_{const12}$), and fluctuating T_a varied from 14°C during the dark phase to 24°C during the light phase ($W_{fluc14-24}$). The constant T_as 24 and 18°C were chosen as the middle between the minimum and maximum of the fluctuating T_a of the respective season (as would be found in a very well insulated resting site). Additionally, a constant T_a of 12°C was included during winter to investigate responses and limitations of *E. telfairi* to a constant temperature regime at the lower end of temperatures in their resting sites. Animals were randomly assigned to the different experimental treatments and were used in multiple experiments. Each treatment lasted for a minimum of two weeks and the sequence of experimental treatments within each season was randomized.

Measurement of Ambient and Skin Temperature

Skin temperature (T_{skin}) and T_a were measured with temperature data loggers (3.3 g; iButton, DS1922L, Maxim Integrated Products, Inc., Sunnyvale, United States) set to logging intervals of 15 min and a resolution of 0.0625°C. The data loggers were taped to the shaved animals' abdominal regions with medical tape (Fixomull stretch, BSN medical, Hamburg, Germany), which did not restrict the tenrecs' movements in any way and remained in close contact to the skin during activity. External temperature loggers give reliable approximation of T_b, especially during resting and torpor phases, when the animals are curled up with the logger positioned inside (Barclay et al., 1996; Dausmann, 2005). When loggers fell off (mainly during activity phases), they were re-taped to the animals without any apparent disturbance before they became torpid again. As the tenrecs (and cages) were checked daily, we found detached loggers within 24 h and the corresponding data were omitted from analyses.

Loggers for recording T_a were fixed to the inside of each cage (to control the preciseness of the climate chamber) and each nest box (T_n in the analyses). Temperature readings were averaged for every hour. As T_{skin} of the tenrecs is very flexible and sometimes low even in the non-torpid state, it was not possible to define a torpor/non-torpor threshold for T_{skin}. However, T_{skin} was always either almost at T_a, or distinctly above it. Thus, the animals were considered to be torpid when T_{skin} was at or only slightly above T_n ($T_{skin}–T_n \leq 2°C$), as confirmed by the obvious drop and increase in MR at the beginning and end of each torpor bout and of each activity phase (summer) or arousal (winter) (see **Figure 1**). The term "arousals" in this study thus includes the (active) rewarming phase as well as periods of activity with normothermic T_{skin} (especially during summer) and parts of the cooling phase.

Measurement of Metabolic Rate

Metabolic rate was measured via oxygen consumption with two differential oxygen analyzers (OxBox, FIWI, Vienna University, Vienna, Austria). Air was drawn at a rate of 50 L/h from the nest boxes, which served as respiratory chambers and were connected to the oxygen analyzers with airtight tubes (Tygon tubing ST, Saint-Gobain, Charny, France). Dividers in the middle of the nest boxes lessened air mixing between ambient air and the inside of the nest boxes. A gas splitter (RM Gas Flow Multiplexer, Sable Systems, Las Vegas, NV, United States) was used to rotate air flow amongst four different nest boxes. Thus, a total of eight animals could be measured at the same time using the two oxygen analyzers. During a 1-h long rotation cycle, all nest boxes were measured for 15 min and data stored every minute, and once per hour reference air was measured for 5 min to obtain a baseline value. Depending on the start of the measurement, the reference air measurement fell into the measurement period of different animals. Air leaving the nest boxes was dried with silica gel (Silica Gel Orange, 2–5 mm, Carl Roth, Karlsruhe, Germany) before entering the analyzer and the flow meter. The oxygen analyzers were calibrated with calibration gas mixtures produced with a gas-mixing pump (2KM300/a, H. Wösthoff GmbH, Bochum, Germany) and values were corrected for the CO_2 effect with: $\Delta Vol\%O_2 = \Delta O2 + 0.0818^{*}\Delta O_2$. Oxygen consumption was calculated using the following equation: $\dot{V}O_2$ [ml $O_2{}^{*}h^{-1}$] = $\Delta Vol\%O_2$ * flow [$L^{*}h^{-1}$] * 10 (Heldmaier and Steinlechner, 1981) and was converted to $\dot{V}O_2$ per gram body mass (ml $O_2{}^{*}g^{-1}{}^{*}h^{-1}$). Mass used in the analysis was calculated as the average of the body mass before and after the experimental periods. Mean hourly values of oxygen consumption were calculated and synchronized with corresponding temperature readings. For each daily torpor bout, hibernation bout, arousal and activity phase minimum, maximum and average oxygen consumption and T_{skin} were calculated. Additionally, the average oxygen consumption for the warming phase of each torpor bout was calculated. Oxygen consumption could only be measured as torpid or resting metabolic rate (RMR) in most cases, as measurements were only possible when the animals were in the nest boxes and therefore total energy budgets, including activity could not be calculated. Due to technical problems, $\dot{V}O_2$ is not available for $W_{const12}$.

Statistical Methods

If not stated otherwise, values are given as mean ± standard deviation, with N denoting the number of individuals tested

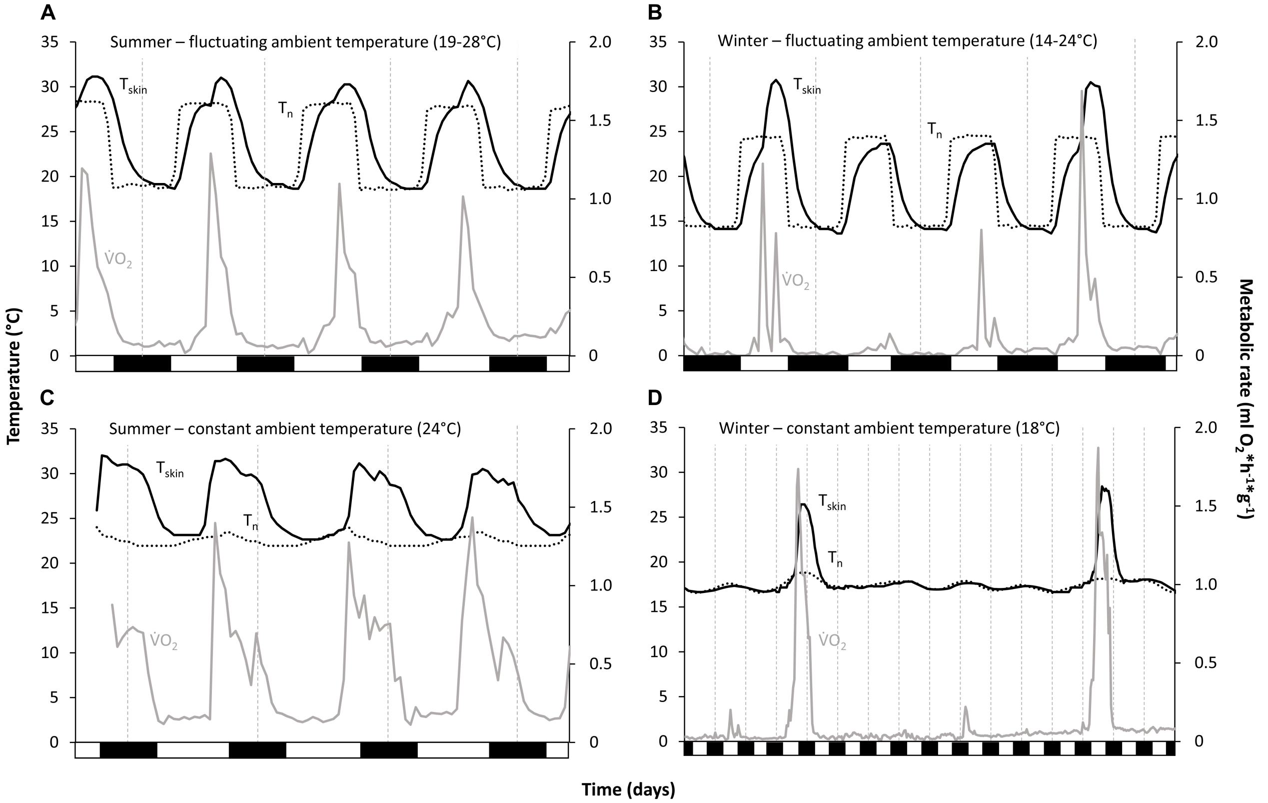


FIGURE 1 | Torpor and arousal/activity phase pattern of *Echinops telfairi* for four experimental treatments. **(A,B)** fluctuating temperature conditions (summer and winter), **(C,D)** constant temperature conditions (summer and winter). Tenrecs became torpid every day; under fluctuating temperature conditions, they used these fluctuations for passively warming from torpor until maximum nest box temperature, whereas under constant temperature conditions they had to rely on endogenous heat production. Black line: skin temperature; dotted line: nest box temperature; gray line: metabolic rate; vertical dashed lines indicate midnight, black boxes on the *x*-axis the scotophase.

per treatment, *n* the number of observations. As the aim of our study was to identify responses on a population level and due to the uneven distribution of sexes within and across treatments, the data from both sexes were pooled. All statistical procedures were done using R (R Development Core Team, 2018). Differences in weight were tested with a *t*-test after the data were checked with a Kolmogorov–Smirnov test for normal distribution. To account for an unbalanced data set, differences in minimum $\dot{V}O_2$ and T_{skin} during torpor, torpor bout duration (TBD), maximum $\dot{V}O_2$, average $\dot{V}O_2$ and T_{skin} during arousal and length of the activity period were tested via generalized estimation equations with a Gaussian error structure and an autoregressive, AR1, correlation structure ("geeglm" in library "geepack," Yan, 2002; Yan and Fine, 2004; Halekoh et al., 2006), followed by type 1 ANOVA. Individual ID was included as a random effect to adjust for repeated measurements. To test for statistical differences of $\dot{V}O_2$, we used total $\dot{V}O_2$ per animal as the response variable and adjusted for body mass by including it as a covariate. Mass-specific metabolic rates are given for descriptive purposes but were not used in statistical analyses. *Post hoc* analyses were performed as Tukey tests ("glht" in library "multcomp," Hothorn et al., 2008).

Rayleigh tests were used to determine whether circular data (timing of arousals) differed significantly from random ("rayleigh.test" in library "circular," Jammalamadaka and Sengupta, 2001). Watson two-tailed tests were performed to test differences between the timing of arousals between treatments ("watson.two.test" in "circular"). To examine differences in the variance of arousal timing, we performed generalized estimation equations as described above. Variance was calculated as the individual deviation from the mean time of arousal start (minutes from midnight) per treatment. Resultant probability values were compared to an α-value of 0.05.

RESULTS

Behavior and Body Mass

Corresponding with their nocturnal lifestyle, all animals were active during the late day and early night throughout summer and used short bouts of torpor during the rest phase on a daily basis. Only one female animal showed an activity phase exceeding 24 h on two occasions during $S_{const24}$ (28 and 40 h long). During winter, all *E. telfairi* hibernated for several months ($N = 27$) and spent most time inside their nest boxes, even during arousals, although some individuals briefly left their boxes to drink. Most animals did not eat during winter (see below). Average body mass during summer was 156 ± 28 g (N = 27). Mean body mass fell to 126 ± 24 g (N = 23) during the winter (t-test, $t = 10.616$, $p < 0.001$).

In a separate study, food intake of the study individuals across the seasons was measured. During winter, most tenrecs did not eat. If they ate, it was only a small fraction of the amount they ate during summer (about 2.7 g mealworms and 1.4 g banana (dry weights) per month in summer vs. 0.2 g mealworms and 0.3 g banana per month in winter (N = 6, n = 18; t-test, $t = -26.393$, $p < 0.001$ for mealworms; t-test, $t = -2.696$, $p = 0.043$ for banana, Lund, 2009).

Torpor Duration, Minimum T_{skin}, and Oxygen Consumption

Torpor bout length (TBD), minimum oxygen consumption ($\dot{V}O_2$), and minimum T_{skin} were significantly different between the five experimental conditions (for data and statistical analyses see **Tables 1**, **2**). During both summer temperature treatments *E. telfairi* entered short bouts of torpor every day during the first part of the resting phase (**Figures 1A,C**). Minimum T_{skin} closely resembled minimum T_n under both summer conditions ($S_{const24}$ and $S_{fluc19-28}$) and hourly mean minimum T_{skin} thus was significantly lower during $S_{fluc19-28}$ than during $S_{const24}$ (**Tables 1**, **2**). Furthermore, TBD was almost double under the fluctuating condition and thus significantly longer than during $S_{const24}$ (**Tables 1**, **2**).

Animals hibernated in all winter conditions. The general hibernation pattern was similar during the $W_{const12}$ and $W_{const18}$ treatments. Animals entered torpor bouts of varying lengths during which T_{skin} was fairly constant and close to T_n, alternating with periodic arousals (**Figure 1B**). Torpor bouts during winter were significantly longer than during both summer conditions (**Tables 1**, **2**). Some torpor bouts were interrupted by the end of the experiment and therefore could have even been longer. Torpor bouts lasted twice as long and significantly longer in $W_{const12}$ as in $W_{const18}$ (**Tables 1**, **2**). In fluctuating winter conditions, T_{skin} closely tracked the T_n cycle during hibernation bouts passively, with the lowest T_{skin} recorded as 10.7°C under $W_{const12}$ (**Figure 1D** and **Table 1**; climate chambers did sometimes deviate a little bit from the set temperature, in this case T_a was slightly cooler than 12°C). Arousals were more frequent in fluctuating than in constant winter temperatures and the hibernation bouts were highly variable in length (range: 6–359 h) and significantly different to the other treatments (except $W_{const18}$; **Tables 1**, **2**). The differences in hourly mean minimum T_{skin} were significantly different for all five experimental groups (**Tables 1**, **2**).

Minimum $\dot{V}O_2$ during torpor was significantly related to the minimum experimental temperature, i.e., lowest at $W_{fluc14-24}$ and $S_{fluc19-28}$ (0.03 ml $O_2{}^*g^{-1}{}^*h^{-1}$ for both) and more than four-fold higher under $S_{const24}$ (0.14 ml $O_2{}^*g^{-1}{}^*h^{-1}$; **Tables 1**, **2**; **Figure 1**). Minimum $\dot{V}O_2$ differed significantly between all treatments except between both fluctuating conditions (**Tables 1**, **2**).

Arousals, Maximum T_{skin}, and Oxygen Consumption

Animals used daily T_n fluctuation for passively rewarming from torpor and only activated endogenous heating after T_{skin} reached the high daytime T_n passively (**Figures 1A,B**). At the end of a torpor bout (which coincided with the start of the active rewarming and the initiation of activity phase), $\dot{V}O_2$ increased sharply and remained high until the end of the activity phase when it quickly dropped again (**Figure 1**). Maximum $\dot{V}O_2$ during arousals or activity phases, average $\dot{V}O_2$ and maximum T_{skin} reached during arousals and the length of the arousal/activity phase differed significantly between the temperature conditions (**Tables 1**, **2**). Maximum $\dot{V}O_2$ and average $\dot{V}O_2$ during arousals were highest for $S_{const24}$ and $W_{const18}$ ($\dot{V}O_2$ max: 1.38 ml $O_2{}^*g^{-1}{}^*h^{-1}$ and 1.50 ml $O_2{}^*g^{-1}{}^*h^{-1}$; $\dot{V}O_2$ ave: 0.62 ml $O_2{}^*g^{-1}{}^*h^{-1}$ and 0.68 ml $O_2{}^*g^{-1}{}^*h^{-1}$; no significant differences between the two for either variable, **Table 2**), where all heating had to be endogenously initiated, and lowest during $W_{fluc14-24}$, where passive heating over most of the T_{skin} increase reduced energy expenditure (0.89 ml $O_2{}^*g^{-1}{}^*h^{-1}$), followed by $S_{fluc19-28}$ (0.95 ml $O_2{}^*g^{-1}{}^*h^{-1}$). While $W_{fluc14-24}$ and $W_{const18}$ were significantly different for maximum $\dot{V}O_2$, the difference between $S_{fluc19-28}$ and $S_{const24}$ was not significant (**Table 2**). All other treatments except $S_{const24}$ and $W_{const18}$ were significantly different from each other (**Tables 1**, **2**). Thus, the high levels of maximum $\dot{V}O_2$ during active heating under constant temperature conditions carried over into average $\dot{V}O_2$ during arousals and activity phases.

$\dot{V}O_2$ during rewarming in the fluctuating temperature conditions (0.45 ml $O_2{}^*g^{-1}{}^*h^{-1}$ in summer and 0.48 ml $O_2{}^*g^{-1}{}^*h^{-1}$ in winter), including passive heating phases, was only about half, and significantly lower than that of the active heating phases observed under the constant conditions which relied exclusively on endogenous heat production (**Tables 1**, **2**). Arousal rewarming $\dot{V}O_2$ during $S_{const24}$ and $W_{const18}$ was significantly higher than $S_{fluc19-28}$ and $W_{fluc14-24}$, respectively (**Tables 1**, **2**) indicating energy saved by the use of passive heating. There were, however, no difference between the two fluctuating conditions ($S_{fluc19-28}$ and $W_{fluc14-24}$) and the two constant conditions ($S_{const24}$ and $W_{const18}$, **Table 2**).

T_{skin} followed the pattern of $\dot{V}O_2$ with a lag time that depended on the experimental condition (**Figure 1**). Hourly mean maximal T_{skin} during arousals was highest for $S_{const24}$ (**Table 1**); the highest overall recorded T_{skin} was 32.8°C. T_{skin} in the other treatments was slightly, but significantly lower (**Tables 1**, **2**). In the $S_{const24}$ condition, the animals maintained elevated T_{skin} for about 14 h per day during the activity phase, but only for about 9 h, and significantly shorter, under $S_{fluc19-28}$, due to the passive heating phase under the fluctuating conditions (**Table 2**). Arousal or activity phases were significantly shortest in the $S_{fluc19-28}$ treatment (about 7 h; **Table 2**) and intermediate in the $W_{fluc14-24}$ treatment (**Figure 2**).

TABLE 1 | Torpor and arousal/activity phase characteristics of *Echinops telfairi* for all five experimental treatments.

		Hibernation/daily torpor bouts			Arousals/activity phases				
Treatment		**Duration (h)**	**T_{skin} min (°C)**	**$\dot{V}O_2$ min (ml O_2 $g^{-1}h^{-1}$)**	**Duration (h)**	**T_{skin} max (°C)**	**$\dot{V}O_2$ average (ml O_2 $g^{-1}h^{-1}$)**	**$\dot{V}O_2$ max (ml O_2 $g^{-1}h^{-1}$)**	**Rewarming $\dot{V}O_2$ (ml O_2 $g^{-1}h^{-1}$)**
$S_{fluc19-28}$	Mean	15.3 ± 0.9^{a}	18.8 ± 0.3^{a}	0.03 ± 0.03^{a}	6.7 ± 1.0^{a}	29.8 ± 1.0^{a}	0.36 ± 0.12^{a}	0.95 ± 0.28^{a}	0.45 ± 0.18^{a}
	Range	11–25	17.7–19.7	0.01–0.14	4–8	27.9–31.7	0.1–0.59	0.21–1.69	0.08–0.87
	N, *n*	*N* = 8, *n* = 36		*N* = 8, *n* = 32	*N* = 8, *n* = 37		*N* = 8, *n*-33		*N* = 8, *n*-33
$S_{const24}$	Mean	8.5 ± 2.1^{b}	23.0 ± 0.6^{b}	0.14 ± 0.04^{b}	14.5 ± 9.9^{b}	31.2 ± 0.7^{b}	0.62 ± 0.19^{b}	1.38 ± 0.63^{a,b,c}	0.93 ± 0.49^{b,c}
	Range	1–14	21.7–25.4	0.01–0.26	4–40	29.1–32.8	0.3–1.05	0.42–3.43	0.14–1.68
	N, *n*	*N* = 6, *n* = 28		*N* = 6, *n* = 25	*N* = 6, *n* = 28		*N* = 6, *n* = 25		*N* = 6, *n* = 13
$W_{fluc14-24}$	Mean	88.1 ± 117.7^{c}	13.3 ± 0.8^{c}	0.03 ± 0.01^{a}	11.8 ± 4.0^{c}	29.4 ± 1.1^{a}	0.26 ± 0.11^{c}	0.89 ± 0.55^{a,c}	0.48 ± 0.27^{a,c}
	Range	6–359	11.0–14.2	0.01–0.05	4–12	24.7–31.4	0.07–0.63	0.11–3.09	0.07–0.82
	N, *n*	*N* = 13, *n* = 43		*N* = 13, *n* = 41	*N* = 10, *n* = 40		*N* = 10, *n* = 39		*N* = 10, *n* = 39
$W_{const18}$	Mean	100.0 ± 51.6^{ce}	17.0 ± 0.4^{d}	0.09 ± 0.07^{c}	9.4 ± 2.3^{c}	28.0 ± 1.1^{c}	0.68 ± 0.26^{b}	1.50 ± 0.64^{b}	1.00 ± 0.41^{b}
	Range	16–194	15.9–18.1	0.01–0.38	3–17	23.5–30.1	0.06–1.12	0.15–2.74	0.43–1.76
	N, *n*	*N* = 18, *n* = 44		*N* = 17, *n* = 29	*N* = 15, *n* = 39		*N* = 12, *n* = 19		*N* = 7, *n* = 18
$W_{const12}$	Mean	204.7 ± 75.3^{d}	11.3 ± 0.3^{e}	n/a	12 ± 7.1†	26.6 ± 0.1†	n/a	n/a	n/a
	Range	83–287	10.7–12.2	n/a	7–17	26.5–26.7	n/a	n/a	n/a
	N, *n*	*N* = 8, *n* = 12		n/a	*N* = 2, *n* = 2		n/a	n/a	n/a

Values are given as mean ± SD. N denotes the number of individuals tested per treatment, n the number of observations. Differing letters indicate statistical differences. †Not enough data for statistical analysis. $S_{fluc19-28}$: summer, fluctuating condition (19–28°C); $S_{const24}$: summer, constant condition (24°C); $W_{fluc14-24}$: winter, fluctuating condition (14–24°C); $W_{const18}$: winter, constant condition (18°C); $W_{const14}$: winter, constant condition (14°C); n/a: not applicable.

TABLE 2 | Statistical parameters for ANOVA and Tukey *post hoc* tests for torpor and arousal/activity phase characteristics of *Echinops telfairi* for all five experimental treatments.

		Hibernation/daily torpor bouts			**Arousals/activity phases**				
Treatment		**Duration (h)**	**T_{skin} min (°C)**	**$\dot{V}O_2$ min (ml O_2 $g^{-1}h^{-1}$)**	**Duration (h)**	**T_{skin} max (°C)**	**$\dot{V}O_2$ average (ml O_2 $g^{-1}h^{-1}$)**	**$\dot{V}O_2$ max (ml O_2 $g^{-1}h^{-1}$)**	**Rewarming $\dot{V}O_2$ (ml O_2 $g^{-1}h^{-1}$)**
ANOVA	df	4	4	3	3	3	3	3	3
	χ^2	183	5007	85.9	54.9	88.8	43.1	8.05	18.09
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.045	<0.001
$S_{fluc19-28}$ vs. $S_{const24}$	*z*	5.45	20.6	5.25	4.01	3.89	3.4	1.89	2.58
	p	<0.001	<0.001	0.001	<0.001	<0.001	0.004	0.225	0.045
$S_{fluc19-28}$ vs. $W_{fluc14-24}$	*z*	2.74	41.4	1.28	4.20	1.16	2.75	0.19	0.94
	p	0.0388	<0.001	0.553	<0.001	0.649	0.028	0.100	0.774
$S_{fluc19-28}$ vs. $W_{const18}$	*z*	9.87	17.0	2.90	5.66	5.51	4.21	3.11	4.35
	p	<0.001	<0.001	0.018	<0.001	<0.001	<0.001	0.010	<0.001
$S_{fluc19-28}$ vs. $W_{const12}$	*z*	7.77	54.5	n/a	n/a	n/a	n/a	n/a	n/a
	p	<0.001	<0.001						
$S_{const24}$ vs. $W_{fluc14-24}$	*z*	3.21	42.0	8.06	3.27	4.93	5.36	1.67	2.20
	p	0.0078	<0.001	<0.001	0.005	<0.001	<0.001	0.333	0.116
$S_{const24}$ vs. $W_{const18}$	*z*	10.65	28.2	2.57	2.65	9.33	1.22	0.95	0.60
	p	<0.001	<0.001	0.046	0.032	<0.001	0.603	0.775	0.928
$S_{const24}$ vs. $W_{const12}$	*z*	7.79	50.1	n/a	n/a	n/a	n/a	n/a	n/a
	p	<0.001	<0.001						
$W_{fluc14-24}$ vs. $W_{const18}$	*z*	2.27	23.2	4.64	2.32	4.52	5.95	2.79	3.80
	p	0.1159	<0.001	<0.001	0.077	<0.001	<0.001	0.0259	<0.001
$W_{fluc14-24}$ vs. $W_{const12}$	*z*	5.35	12.5	n/a	n/a	3.85	n/a	n/a	n/a
	p	<0.001	<0.001			<0.001			
$W_{const18}$ vs. $W_{const12}$	*z*	4.35	38.9	n/a	n/a	3.23	n/a	n/a	n/a
	p	<0.001	<0.001			0.0087			

$S_{fluc19-28}$: summer, fluctuating condition (19–28°C); $S_{const24}$: summer, constant condition (24°C); $W_{fluc14-24}$: winter, fluctuating condition (14–24°C); $W_{const18}$: winter, constant condition (18°C); $W_{const14}$: winter, constant condition (14°C); n/a: not applicable.

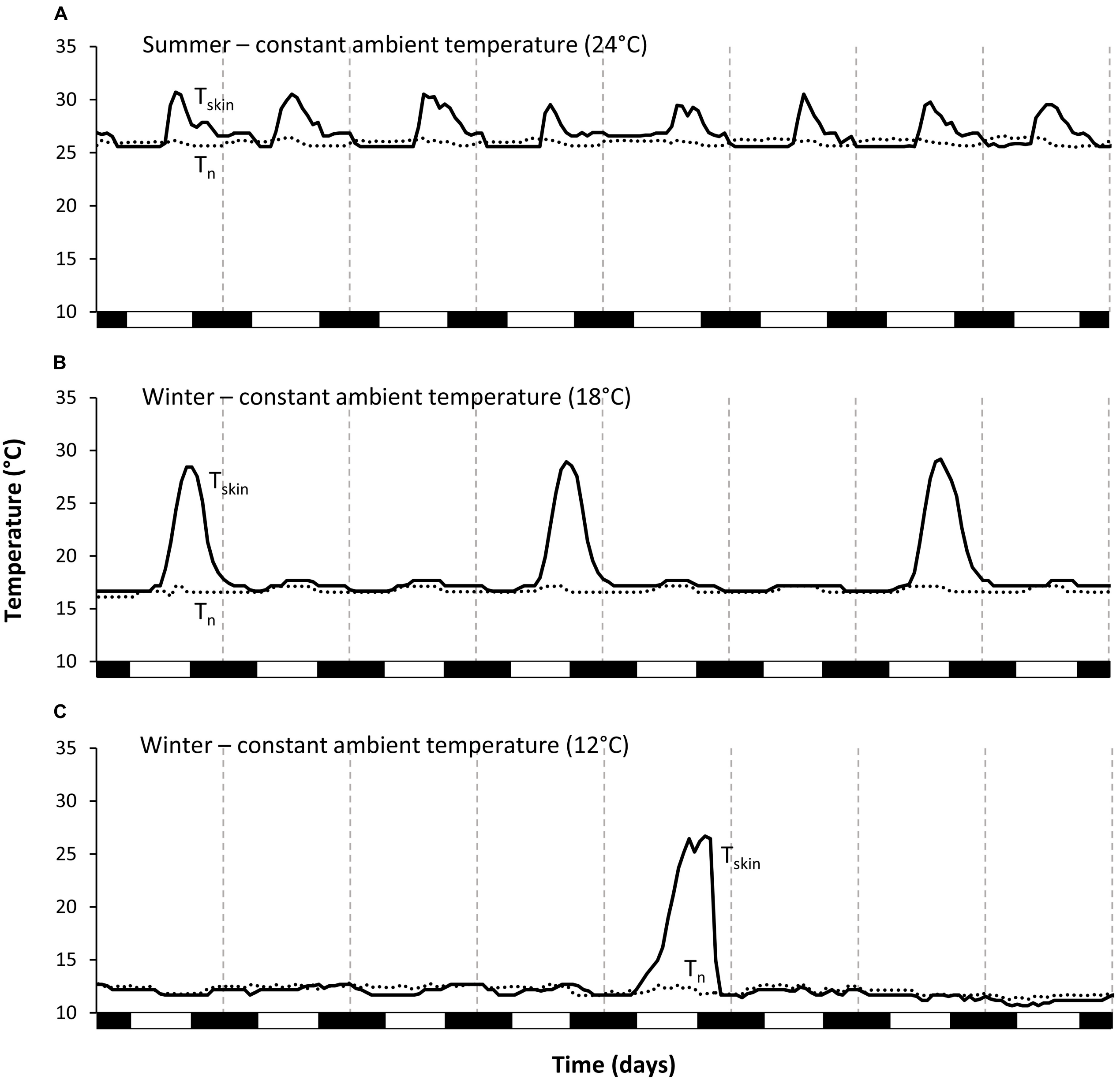
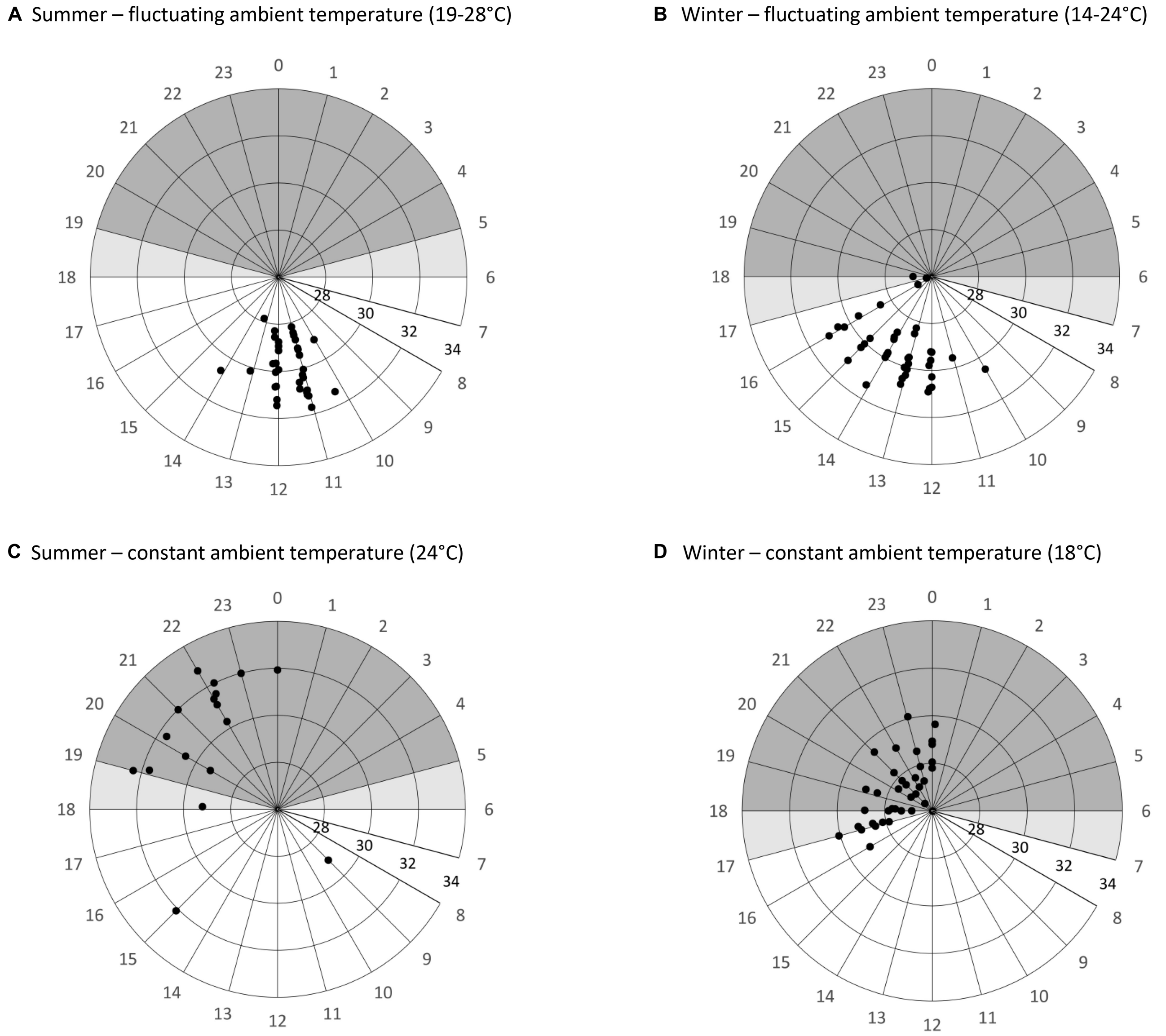


FIGURE 2 | Examples of typical sequences of skin temperature of one individual *Echinops telfairi* during the different constant treatments over eight days. **(A)** Summer 24°C, **(B)** winter 18°C, and **(C)** winter 12°C. During summer, tenrecs aroused daily to normothermic skin temperature; during winter, torpor bouts became longer, even more so at the lower ambient temperature. Black line: skin temperature; dotted line: nest box temperature; vertical dashed lines indicate midnight, black boxes on the *x*-axis the scotophase.

The timing of the arousals differed significantly from a random distribution for all treatments (**Figure 3**). We excluded W_{cost12} from arousal data analysis as only two full arousals were recorded. However, there were three unsuccessful attempts at rewarming under this condition (**Figure 4**), which were never observed in any other treatment. In summer, animals under $S_{const24}$ started to rewarm at 18:43 ± 02:24 h ($N = 6$, $n = 28$; Rayleigh test: $r = 0.7372$, $p < 0.001$) and reached their maximum T_{skin} about 2 h later. Animals under $S_{fluc19-28}$ used daily T_n fluctuation for passively rewarming from torpor and only activated endogenous heating on average at 11:34 ± 00:35 h ($N = 8$, $n = 37$; Rayleigh test: $r = 0.978$, $p < 0.001$; **Figure 3**), which was significantly earlier than under the constant summer treatment (Watson's test: $x = 1.3308$, $p < 0.001$; **Figure 3**).

During winter, arousals were less frequent, and even more so during the constant treatments. During $W_{const12}$ only two arousals were recorded and those varied considerably in timing (15:00 and 22:00 h). There was considerable variation in


A Summer – fluctuating ambient temperature (19-28°C)

B Winter – fluctuating ambient temperature (14-24°C)

C Summer – constant ambient temperature (24°C)

D Winter – constant ambient temperature (18°C)

FIGURE 3 | Timing of arousals (initiation of endogenous heat production) and maximum skin temperature during arousals/activity phases of *Echinops telfairi* for four experimental treatments **(A–D)**. Data shown indicate the time of day (degrees) and level of maximum skin temperature (°C; radial distance). Shaded areas illustrate the scotophase, lighter shaded areas the transitional times between night and day phase. Under fluctuating conditions **(A,B)**, arousals are more synchronous and initiated when T_{skin} passively reaches maximal T_{n} well before the beginning of the scotophase. Under constant conditions **(C,D)**, the start of arousals is scattered over a longer time period, but almost always only after the beginning of the scotophase. Maximal T_{skin} is highest during the activity phases under constant summer condition (24°C), and lowest and least variable during the arousals under constant winter conditions (18°C).

the timing of arousals under $\text{W}_{\text{const18}}$. Arousals for $\text{W}_{\text{const18}}$ started at 19:54 ± 04:27 h (N = 16, n = 40; Rayleigh test: $r = 0.896$, $p < 0.001$) and animals needed between four to 6 h to rewarm. For $\text{W}_{\text{fluc14-24}}$ arousals started at 13:51 ± 01:20 h, after T_{skin} had passively reached high daytime T_{n} (N = 10, n = 41; Rayleigh test, $r = 0.896$, $p < 0.001$; **Figure 3**). Arousals started more synchronously during both fluctuating treatments than under constant T_{a}, i.e., the start time of the arousals was less variable (ANOVA, df = 3, $\chi^2 = 51.3$, $p > 0.001$; $z > 2.82$, $p < 0.0212$; **Figure 3**); while arousals were even more synchronized during S_{fluc24} than W_{fluc18} (deviation from mean 0.43 ± 0.35 min (N = 9, n = 38) and 1.06 ± 0.82 min (N = 10, n = 41), respectively; $z = 4.08$, $p > 0.001$), there were no significant differences between the two constant treatments (deviation from mean $\text{S}_{\text{const24}}$ 1.62 ± 0.79 min (N = 7, n = 29) and $\text{W}_{\text{const18}}$ 2.43 ± 1.72 min (N = 16, n = 40); z = 0.20, $p = 0.9970$). In general, in all analyzed parameters, $\dot{\text{V}}\text{O}_2$ was always higher in the constant than in the fluctuating conditions (**Table 1**).

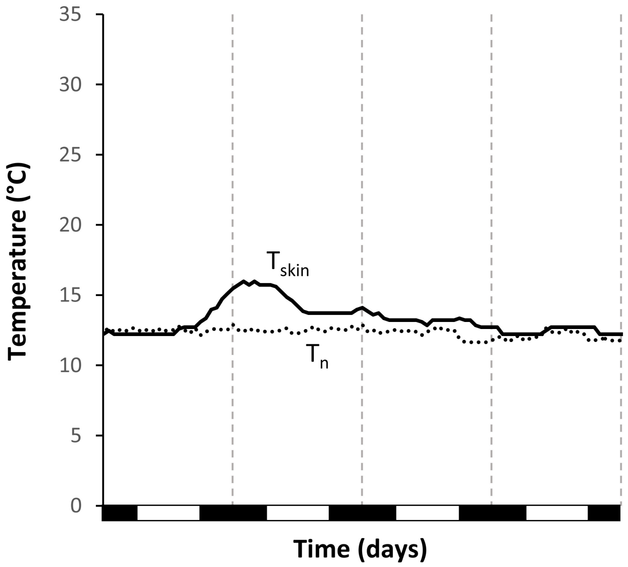


FIGURE 4 | Unsuccessful attempt at rewarming from torpor of *Echinops telfairi* under constant temperature treatment in winter ($W_{const\,12}$). During this event, skin temperature reached a maximum of only 14.7°C and only slowly returned to the level of nest box temperature over two days. Black line: skin temperature; dotted line: nest box temperature; vertical dashed lines indicate midnight, black boxes on the x-axis the scotophase.

DISCUSSION

Temperature regime was found to have a significant effect on hibernation patterns of the highly heterothermic *E. telfairi* highlighting the importance of incorporating realistic temperatures in the study of hibernation. The tenrecs entered torpor every day and fluctuating temperatures acted as a *zeitgeber* that allowed for more synchrony in activity between individuals as well as lower costs of arousal from torpor. Under fluctuating summer T_a ($S_{fluc19-28}$) conditions, *E. telfairi* was able to lower its $\dot{V}O_2$ during daily resting phases to the same low levels as during winter hibernation bouts under fluctuating conditions (both 0.03 ml $O_2{}^*g^{-1}{}^*h^{-1}$). In both cases torpid metabolic rates were much lower than would be expected from mere Q_{10} effects when comparing torpid $\dot{V}O_2$ with maximum $\dot{V}O_2$ at maximum T_{skin} (0.95–0.89 ml $O_2{}^*g^{-1}{}^*h^{-1}$; decrease in T_b from about 30°C to about 20°C and drop in $\dot{V}O_2$ to less than 5% of resting rates, instead of about 50% predicted by a Q10 of 2–3; **Table 1**). Thus, during the resting phases of fluctuating summer conditions, the decrease in $\dot{V}O_2$ was not "only" due to the suppression of thermogenesis leading to the highly labile thermoregulation tenrecs are famous for (Nicoll, 1986; Stephenson and Racey, 1994; Lovegrove and Génin, 2008; Levesque et al., 2014), but also an indication of active metabolic inhibition. Only one female, in $S_{const24}$, remained normothermic for longer than 24 h on two occasions. Furthermore, under the constant summer T_a ($S_{const24}$), elevated environmental temperatures precluded the attainment of the lowest levels of torpid $\dot{V}O_2$, however, $\dot{V}O_2$ was still lower than would be expected by Q_{10} effects alone (from 1.38 to 0.14 ml $O_2{}^*g^{-1}{}^*h^{-1}$; about 10% of RMR). However, at least during summer, the tenrecs needed temperature fluctuations, which included lower temperatures, to take advantage of the lowest, most energy saving levels of torpid $\dot{V}O_2$. In general, fluctuating temperature conditions proved to be energetically more efficient for *E. telfairi*. As temperatures are predicted to continue to increase with global climate change, this could pose a significant increase in energetic costs during torpor (Lovegrove et al., 2014a).

Despite often occurring at higher T_a (but not always, see Nowack et al., 2020), tropical hibernation and daily torpor has the potential to realize significant energy savings as well as its beneficial effect on water usage (Cooper et al., 2005; Withers et al., 2012). *E. telfairi* in our study reached minimal levels of metabolism during torpor episodes comparable to that of temperate and arctic species (0.03 ml $O_2{}^*g^{-1}{}^*h^{-1}$, Heldmaier et al., 2004). However, as active metabolic rates of tenrecs are lower, the relative energy savings are less pronounced. Apparently, this level of torpid metabolism seems to be at a threshold for mammalian minimal MR (see Frappell and Butler, 2004). The average torpid $\dot{V}O_2$ of *E. telfairi* was 0.06 ml $O_2{}^*g^{-1}{}^*h^{-1}$, the same as in the Malagasy lemur *C. medius* (Dausmann et al., 2009), and the greater hedgehog tenrec (0.08–0.1 $O_2{}^*g^{-1}{}^*h^{-1}$, Levesque and Lovegrove, 2014), indicating a general, or at least Malagasy, level for tropical hibernation. When *E. telfairi* were hibernating under the fluctuating T_a regime, $\dot{V}O_2$ correlated with T_a and was lower during the colder night phase compared to the warmer day phase. Together with the longer duration of hibernation bouts at lower T_a and thus fewer, energetically costly arousals, it is likely that it is energetically favorable for *E. telfairi* to hibernate at lower T_a, at least down to a certain limit (see below).

In general, tenrec species have been reported to be highly heterothermic, except when they are pregnant or lactating (Stephenson and Racey, 1993a,b; Poppitt et al., 1994;

Levesque and Lovegrove, 2014; Levesque et al., 2014). In our study individuals were not reproducing and became or continued to be torpid every day throughout the study. Flexible thermoregulation reduces general energy expenditure, however, also limits the ambient temperature breadth, over which an organism can function (Treat et al., 2018). Torpor, on the other hand, might counterbalance this disadvantage, broadening the temperature niche. Indeed, torpor use has been found to lower the risk of extinction in highly variable and quickly changing environments (Geiser and Turbill, 2009; Liow et al., 2009). Maximal T_{skin} during activity and arousal phases was similar in the two summer treatments and the two warmer winter treatments, and was in the same range as body temperature reported for intraperitoneally implanted temperature loggers by Lovegrove and Génin (2008), emphasizing the validity of our T_{skin} measurements. Maximal T_{skin} was lowest during the two arousals in the W_{const12} conditions, suggesting limitations of the endogenous heating capacities at constantly low temperatures. In addition, there were also some "unsuccessful" arousals during W_{const12} conditions, suggesting a lower limit for active arousal at around this temperature. Indeed, there seems to be a lower thermal limit for hibernation in *E. telfairi*. Support for this comes from Scholl (1974), who noted that *E. telfairi* was not able to arouse successfully at a T_a of 11°C. This sets an ultimate lower limit of T_a for long-term survival in this species and restricts its potential habitats. Although temperatures do drop below 10°C within the range of *E. telfairi* during winter nights, this does not occur very frequently and will be buffered even in hibernacula with low insulation capacity. More importantly, even on those coldest days, T_a will usually increase above 20°C during the day (Dausmann and Blanco, 2016), ensuring passive rewarming (contrary to Scholl, 1974).

It has been proposed that high costs of rewarming from torpor could limit the efficient employment of heterothermy, reducing energy savings achieved during torpor episodes (Wang, 1979; Humphries et al., 2003), especially for short torpor bouts. However, most terrestrial animals do not live in constant environments, but experience daily and seasonal fluctuations in T_a (Dillon and Woods, 2016; Dillon et al., 2016). Depending on the type and the insulation properties of their resting sites, environmental temperature fluctuations also translate into variable temperatures within the resting sites (Dausmann et al., 2004; Turner, 2020). These fluctuations in T_a can be used for assisted warming from torpor, especially in species living in tropical areas, where daily maximal temperatures tend to be higher than potential torpid T_b set-points, particularly during the winter seasons. Exogenous, mainly passive heating is known from several tropical heterotherms (e.g., *Microcebus murinus*, Ortmann et al., 1997; *Sminthopsis macroura*, Lovegrove et al., 1999; *Elephantulus myurus*, Mzilikazi et al., 2002; Geiser and Drury, 2003; *C. medius*, Dausmann et al., 2009; Thompson et al., 2015), and makes rewarming comparatively inexpensive with 60–85% reductions when compared to active warming (Lovegrove et al., 1999; Schmid et al., 2000; Geiser and Drury, 2003; Warnecke et al., 2008). The benefit from passive heating, either by daily fluctuations of T_a or by radiant heat, might lead to laboratory studies underestimating the energetic advantage of torpor in free-ranging mammals (Mzilikazi et al., 2002), might explain why daily torpor is common in sunny regions and might occur more frequently at low latitudes than hitherto believed (Geiser and Drury, 2003).

Previous laboratory studies with *E. telfairi* have used constant T_a for their experiments (Scholl, 1974; Poppitt et al., 1994; Künzle, 1998; Künzle et al., 2007; Oelkrug et al., 2013). In our study, fluctuating T_a treatments mimicked natural conditions. Under these conditions, *E. telfairi* also used daily T_a fluctuations for passively warming from daily torpor in summer as well as during arousals between hibernation bouts in winter. In both seasons, $\dot{V}O_2$ during rewarming from torpor under the intermediate, constant conditions was about double that of fluctuating temperature indicating an energetic advantage of passive heating. Under fluctuating conditions, the animals only activated endogenous heat production and became active after T_{skin} reached the high daytime T_n passively around noon, as evident by the sharp rise in MR above this threshold. T_a cycles lead to more uniform and synchronized T_{skin} patterns. In this way, T_a cycles not only help rewarming, but also synchronize the activity phases and torpor bouts of the animals, in contrast to constant T_a conditions where there was considerable variation in timing. In addition, arousals started about 6 h later under constant conditions, shortly after the lights had gone off. Possibly, for animals under constant temperature conditions light, or rather darkness, was taken as a (less stringent) cue to initiate exogenous heating.

The timing of torpor is not only important for its effective use (Körtner and Geiser, 2000), but synchronization of activity patterns could also be essential for social interactions, successful foraging bouts, and other important activities. Additional external stimuli, such as photoperiod, also affect the timing of torpor (Heldmaier et al., 1982, 1989; Aujard et al., 1998), but in the field these are often coupled to T_a. Furthermore, some species (e.g., the small marsupial dunnarts, *Sminthopsis* sp.) have been shown to be insensitive to photoperiodic cues (Holloway and Geiser, 1996). A study on *E. telfairi* in an enclosure in Madagascar with natural climatic parameters by Lovegrove and Génin (2008) also found that the tenrecs used passive exogenous heating by T_a before initiating active heat production. Interestingly, they only found daily and prolonged torpor (maximum of 4 days), but not longer hibernation bouts, possibly due to the timing of the study (beginning of winter), constant supply of food, or lack of suitable hibernacula (tree hollows) in the enclosure. In our study, *E. telfairi* hibernated for several months in constant, as well as in fluctuating T_a during the hibernation season (winter). Our results show that T_a cycles can be an effective *zeitgeber* for activity and thermoregulatory rhythms, even during hibernation, and that careful consideration should be given to the choice of temperature regime under laboratory conditions.

During deep hibernation, arctic and temperate species typically display hibernation bouts of about or exceeding two weeks in length (Heldmaier et al., 2004), possibly because of the usually constant, low temperatures that arctic animals are exposed to in their hibernacula (Arnold et al., 1991; Buck and Barnes, 1999). Hibernation bout length is more flexible in tropical hibernators. Some species can exhibit very brief hibernation bouts (Reher et al., 2018), whereas mouse and dwarf lemurs can hibernate for several months without arousals, if they use poorly insulated hibernacula and T_b fluctuates passively with T_a above 30°C at least every couple of days, thus forgoing the need for active arousals with endogenous heating (Dausmann, 2014). Interestingly, fluctuating T_a triggered more frequent arousals during hibernation in *E. telfairi* in our study, therefore reducing average hibernation bout length from over eight ($\mathrm{W}_{\mathrm{const12}}$) and four ($\mathrm{W}_{\mathrm{const18}}$), to about 3.5 days. It is possible that the maximum $\mathrm{T}_{\mathrm{skin}}$ that could be attained passively during the fluctuating T_a treatment was not high enough to satisfy physiological demands and thus to be settled during active arousals. This suggests a threshold temperature, below which hibernators have to actively terminate hibernation bouts after a certain time and which may vary between species, individually as well as temporally (Körtner and Geiser, 2000; Dausmann et al., 2005; Turbill et al., 2008; Lovegrove et al., 2014b). An increase in length of hibernation bouts with decreasing T_a has also been found in temperate and arctic species, e.g., the golden-mantled ground squirrel *Callospermophilus saturatus* (Geiser and Kenagy, 1988), suggesting that this temperature-dependence in hibernation bout length is universal in tropical as well as temperate and arctic hibernators. We found hibernation bout lengths comparable or even above those described by Scholl (1974) for *E. telfairi*, but contrary to the study by Lovegrove and Génin (2008), hibernation was maintained for several months. Interestingly, the only T_b available from a free-ranging tenrec (*Setifer setosus*) hibernating in variable ambient temperatures showed no evidence of periodic arousals, although in that study tree hole temperature did not drop below 18°C (Levesque et al., 2014).

As environmental temperature variations are the norm, rather than the exception (Dillon et al., 2016), this highlights the importance of incorporating temperature variability in laboratory evaluations of animal thermoregulation. The importance of incorporating realistic temperature variability in laboratory physiology has been receiving increasing attention as we seek to predict the effects of increasingly varying climates on animal survival (Vasseur et al., 2014; Dillon and Woods, 2016; Levesque et al., 2016). Our findings show that fluctuating T_a cycles not only affect the timing of arousals and the duration of hibernation bouts in the tropical hibernator *E. telfairi*, but also have an impact on the timing and shape of activity phase and torpor bouts during the non-hibernation season, as well as dramatically influencing energy expenditure in all seasons. Therefore, attempts to understand the energetics and thermoregulation of hibernators would be best served by the inclusion of more realistic temperature cycles to provide a true understanding of the conditions faced by heterotherms in the wild, especially under tropical conditions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Behörde für Gesundheit und Verbraucherschutz (BGV).

AUTHOR CONTRIBUTIONS

KD and JW conceived the idea and carried out the experiments. All authors contributed to the analyses of the data. JN performed the statistical analyses. KD, DL, and JN wrote the manuscript.

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Sex-Specific Response to Caloric Restriction After Reproductive Investment in *Microcebus murinus*: An Integrative Approach

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In seasonal environments, males and females usually maintain high metabolic activity during the whole summer season, exhausting their energy reserves. In the global warming context, unpredictability of food availability during summer could dramatically challenge the energy budget of individuals. Therefore, one can predict that resilience to environmental stress would be dramatically endangered during summer. Here, we hypothesized that females could have greater capacity to survive harsh conditions than males, considering the temporal shift in their respective reproductive energy investment, which can challenge them differently, as well as enhanced flexibility in females' physiological regulation. We tackled this question on the gray mouse lemur (*Microcebus murinus*), focusing on the late summer period, after the reproductive effort. We monitored six males and six females before and after a 2-weeks 60% caloric restriction (CR), measuring different physiological and cellular parameters in an integrative and comparative multiscale approach. Before CR, females were heavier than males and mostly characterized by high levels of energy expenditure, a more energetic mitochondrial profile and a downregulation of blood antioxidants. We observed a similar energy balance between sexes due to CR, with a decrease in metabolic activity over time only in males. Oxidative damage to DNA was also reduced by different pathways between sexes, which may reflect variability in their physiological status and life-history traits at the end of summer. Finally, females' mitochondria seemed to exhibit greater flexibility and greater metabolic potential than males in response to CR. Our results showed strong differences between males and females in response to food shortage during late summer, underlining the necessity to consider sex as a factor for population dynamics in climate change models.

Keywords: caloric restriction, sex, season, reproductive investment, oxidative stress, non-human primate

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INTRODUCTION

In highly seasonal environments, reproduction has evolved to be restrained to the most energetically favorable period to optimize young growth (Fournier et al., 1999), which is a fragile and high-stake phase though crucial to ensure population renewal (Wells, 2000). But with more frequent climatic disturbances related to global warming, there is no longer certainty that summer will support such energetic demands (Canale and Henry, 2010). This season might therefore become a risky period, where animals, exhausted after their reproductive effort, would be more vulnerable to environmental change and become unable to compensate unexpected altered food availability. Additionally, individuals forage to fill up their energy storages in late summer to face winter's own harsh conditions, such as dryness and cold temperatures (Dammhahn and Kappeler, 2008), but could face instead their physiological limit to prolonged environmental stress.

In most mammal species, males and females are invested in specific physiological tasks regarding reproduction, as males produce high quantities of spermatozoa, whereas females ovulate, complete gestation, and lactation. With a large panel of peculiarities in the animal kingdom, from mating strategies to young care sharing or even hermaphroditism, sex-specific behavior and physiology can describe little to huge dimorphism amongst species (Trivers, 1972; Zeveloff and Boyce, 1980). Additionally, seasonality could have contributed to establish a phenological shift in the energetic investment for reproduction between males and females. Indeed, males use their fat stocks for spermatogenesis, a long-standing and costly process (Dewsbury, 1982; Thomsen et al., 2006), obligating males to trigger the production of sperm by anticipation of the mating season, when food availability is still low. In contrast, females mostly invest afterwards, when the environment is energetically favorable (Trivers, 1972; Zeveloff and Boyce, 1980). The ecological "niche" in which animals enter an active metabolic status can thus drastically differ between sexes (Shine, 1989) and could expose males and females to unequal challenges. Moreover, there is growing evidence of sex-specific physiological responses to poor food conditions in various species, which are generally hinged on the reproduction event. Heterothermic females can express hypometabolism in anticipation of the mating season (Humphries et al., 2003; Jonasson and Willis, 2011; CZenze et al., 2017), during gestation and lactation (Lennox and Goodship, 2008; McAllan and Geiser, 2014), or present general self-preserving abilities (Rodriguez-Cuenca et al., 2002; Valle et al., 2007). In a context of more frequent unpredictable climatic disturbances, we ask the question of a sex-imbalance in the trade-off between reproduction and survival and consider the hypothesis of a greater exhaustion in males than in females at the end of summer.

The gray mouse lemur (*Microcebus murinus)* can provide experimental evidence for a sex-biased response to food shortage in summer, as it is a highly seasonal Malagasy primate, able to enter deep winter torpors (Genin and Perret, 2003; Giroud et al., 2008). Although this species is considered monomorphic, males and females express strong differences in their seasonal weight curves, as males begin to lose body mass in the middle of winter concomitantly to testicular growth (Perret and Aujard, 2001; Terrien et al., 2017) but females keep their fat storage until summer when mating takes place. Male mouse lemurs show unusual extreme large testes relative to their body size and display characteristics associated with sperm competition (high spermatogenic efficacy and motility and low percentages of defect) (Harcourt et al., 1981; Aslam et al., 2002). These features are likely to demand strong energy expenditure (Dewsbury, 1982; Wedell et al., 2002; Thomsen et al., 2006), especially since males continue to maintain sexual activity late during the 6 months long-day period (Perret and Aujard, 2001).

A previous food shortage experiment showed that male mouse lemurs can maintain their body mass under a 40% caloric restriction during short day exposure, by increasing their torpor depth and duration, while the same treatment during long days induced mass loss (Giroud et al., 2008). In another study, a 40% food restriction during summer altered learning abilities of males, which surprisingly showed increased resting metabolic rate and no change in activity patterns (Villain et al., 2016). No experimental study has yet formally compared physiological responses to food shortage between sexes, although females were already described to use deeper and longer torpor than males to face food rarefaction at the onset of winter season in field conditions (Vuarin et al., 2015). Females are receptive to copulation for a short period of a few days during the estrus at the vernal equinox in natural conditions or after the photoperiodic change to long-days in breeding colonies (Perret and Aujard, 2001). Moreover, they can maintain their body mass during gestation with small variation of food intake in captive conditions (Wrogemann et al., 2001; Canale et al., 2012a), and are also able to use torpor during gestation but lose weight when lactation occurs regardless of the feeding regimen (Canale et al., 2012b). The reproductive resilience of mouse lemur females to food shortage, i.e., their ability to restore reproductive success after an environmental pressure, seems to remain during the flexible period of lactation, which only impacts litter growth in restricted individuals, while reproductive success stays unchanged compared to *ad-libitum* mothers (Canale et al., 2012a). No field data comparing sexes allows us however to infer about a possible energy exhaustion of males at the end of summer in natural conditions. But we do observe a sex-imbalance in body mass in the breeding colony of Brunoy at this particular period, though animals remain under normal feeding regimen (94.5 ± 2.24 g vs. 86.7 ± 1.99 g for females and males, respectively; $p < 0.001$; unpublished data). This observation strengthens the hypothesis of a rougher summer for males (although animals are not energetically exhausted), even in the absence of environmental perturbations.

For such heterogeneous physiological events occurring over the 6-months period of summer, males and females would likely express variable metabolic statuses and efficacy in their response to an unpredicted environmental stress. We thus tested the occurrence of sex-variability in energy management and its physiological impact on the well-suited model of *M. murinus*. We monitored six males and six females over a period of 2 weeks during which they experienced a 60% caloric restriction (CR) induced at the end of summer after all reproductive events. In an integrative and comparative (females vs. males) approach, we measured various energy-management parameters at different scales, from the whole organism (respiratory metabolism using indirect calorimetry, metabolic and sexual hormones) to the cellular level (mitochondrial respiration of cultured fibroblasts) and stress-related indicators (urinary and blood markers of oxidative damage and anti-oxidant activity) to better describe the physiological mechanisms modulated by CR from perception of the stress, to response and coping. We expected evidence of a higher impact of CR on males with different metabolic strategies of coping expressed between sexes.

MATERIALS AND METHODS

Tested Animals and Ethical Concerns

Twelve gray mouse lemurs (*M. murinus*), 6 males and 6 females all aged from 1 to 5 years (mean age ± sd: 2 ± 1.1 years) and raised in good health in the breeding colony of Brunoy (MNHN, France, license approval n° E91-114-1), were included in the experiment. All females had just successfully raised a litter of three juveniles, which were weaned before starting the experiment, after 2 months of maternal care. All males had undergone at least one reproduction event, in the presence of one female and one or several males. These animals were thus tested at the late end of the summer-like reproductive season, after 5 months of long photoperiod exposure (14 h light/day), at the time when both sexes reached their minimal body weights (Perret and Aujard, 2001). Animals were kept in individual cages in semi-isolation from the others (visual, hearing and odorant interactions remaining possible between individuals) in climatic chambers for the duration of the experiment (1 month). Temperature and humidity were maintained constant (24–26°C and 55%, respectively). The lemurs were fed with a fresh mixture (egg, concentrate milk, cereals, spicy bread, cream cheese, and water), banana, and were provided with *ad libitum* water. All described experimental procedures were approved by the Animal Welfare board of the UMR 7179, the Cuvier Ethics Committee for the Care and Use of Experimental Animals of the Muséum National d'Histoire Naturelle and authorized by the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (n°14075-2018031509218574) and complied with the European ethic regulations for the use of animals in biomedical research.

Experimental Design

Individuals were designed to be their own control as their physiology in late summer is supposedly not varying as long as the environment remains stable (photoperiod, temperature, food availability) (Perret and Aujard, 2001). We performed an integrative description of physiological parameters, general and cellular scaled, to decipher the energy balance of the animals, their metabolic activity, and their oxidative regulation and damage. For this, animals were fed daily with a control ration (Control treatment "CTL") of 22 g of mixture and 3 g of banana (24.48 kcal.day^{-1}) for 1 week and measured during four consecutive days for indirect calorimetry (Oxymax, Columbus Instrument Inc., Columbus, Ohio, USA). At the end of this procedure, animals went through a series of non-invasive (urine) and invasive sampling (blood puncture via the saphenous vein, and abdominal skin biopsy after lidocaine injection to ensure local analgesia), always performed ∼3 h prior to lights off. Urine samples were used to measure creatinine, cortisol, and 8-OHdG; blood samples were used to measure glycaemia, thyroxin hormone (T4) and antioxidant activity. Finally, skin biopsies were dedicated to develop primary fibroblastic cultures to assess mitochondrial activity. After 1 day of recovery, animals were fed daily with a 60% reduced ration compared to the CTL condition (Caloric Restriction "CR," 9.79 kcal.day^{-1}) for 2 weeks, inducing a sufficient caloric stress to mimic food shortage, but not as much to put animals at risk considering this time of the year where body weights can be low. They were monitored with weighing three times a week to follow their body mass ("BM" in g). At the end of the treatment, they underwent the same procedures as the control period (4-days indirect calorimetry, urine/blood/biopsy sampling). Animals returned under control diet for several days to allow a full recovery from the CR treatment and returned to the breeding colony in their original social groups.

Indirect Calorimetry

Animals were put in monitored cages for 4 consecutive days to measure their consumption of O_2 ("VO_2" in ml.kg.h^{-1}, see equation 1, Lighton, 2018) and production of CO_2 ("VCO_2" in ml.kg.hr^{-1}, see equation 2, Lighton, 2018) in a continuous way using an automated calorimetric set-up (Oxymax, Colombus Instruments Inc., Columbus, Ohio, USA).

$$VO_2 = \frac{FR_i\left[\left(F_iO_2 - F_e{}'O_2\right) - F_e{}'O_2\left(F_e{}'CO_2 - F_iCO_2\right)\right]}{\left(1 - F_e{}'O_2\right)\ m} \tag{1}$$

FR_i and FR_e are the incurrent and excurrent flow rates of all the separate gas species.

F_iO_2 (F_iCO_2) and F_eO_2 (F_eCO_2) are the fractional concentrations of the incurrent and excurrent O_2 (or CO_2).

F_e'O_2 (F_e'CO_2) are the fractional concentrations of excurrent O_2 (CO_2), scrubbed of water vapor. *m* is the body mass of the individual.

$$VCO_2 = \frac{FR_i\left[\left(F_iCO_2 - F_e{}'CO_2\right) - F_e{}'CO_2\left(F_e{}'O_2 - F_iO_2\right)\right]}{\left(1 - F_e{}'CO_2\right)\ m} \tag{2}$$

Air was dried with magnesium perchlorate columns prior to analysis. As metabolic rate ("MR") is acknowledged to be directly linked to oxygen consumption rate (Geiser et al., 2014) we used the VO_2 parameter to describe variations in mouse lemurs' metabolism. Respiratory Exchange Ratio ("RER") was calculated as the ratio of VCO_2/VO_2. This parameter allows to extrapolate the nature of energy substrates used for oxidative metabolism as described in Lusk's oxidation table (Lusk, 1924), RER varying between 1 (indicating a full carbohydrate energy substrate) and 0.7 (obtained when animals only depend on fat energy substrate). Energy expenditure ("EE," kcal.h^{-1}) was obtained as follows: EE $= (3.815 + 1.232 \times \text{RER}) \times VO_2 \times \text{BM}$, an equation that also derives from Lusk's table (Lusk, 1924). Analyses were based on mean parameters over day (period under artificial light) and night (period without artificial light). Maximum and minimum values of each parameter ("Max" and "Min") were also obtained using the *changepoint* package *v2.2.2* (Killick and Eckley, 2014), to detect the significant maximum and minimum changes of the parameters transformed as time series. Time ("hMax" or "hMin" in hh:mm) at which Max and Min values were achieved was also analyzed and set as polar coordinates to be represented in 24 h circular diagrams.

Urine Samples: Cortisol, 8-OHdG, 17-Beta-Estradiol and Testosterone

Cortisol (ng.ml^{-1}; Cortisol Urine ELISA from LDN®, ref MS E-5100) and 8-hydroxy-2'-deoxyguanosine ("8-OHdG," in ng.ml^{-1}; OxiSelect™ Oxidative DNA Damage Elisa kit, Cell Biolabs Inc., ref STA-320) were measured in duplicates from urine samples as indicators of the organisms' response to environmental stress (Miller et al., 1991) and oxidative-stress related DNA damage (Loft et al., 1993), respectively. 17-beta-Estradiol ("17-beta-Estradiol" in pg.ml^{-1}; 17beta-Estradiol, IBL, ref RE52041) and Testosterone ("Testosterone" in ng.ml^{-1}; Testosterone ELISA kit from Abcam, ref ab108666) were also quantified in urine. Creatinine concentration (mg.ml^{-1}) was used to normalize all urine measurements as an indicator of renal filtration activity (Microvue™ Creatinine Elisa kit, Quidel® Corporation, ref 8009). Results are thus expressed in ng.mg Creat.$^{-1}$ or pg.mg Creat.$^{-1}$.

Blood Samples: Fasting Glycaemia, Thyroxin Hormone, and Antioxidant Machinery

Fasting glycaemia (mg.dl^{-1}) was directly obtained at animals' bedside during blood sampling with a non-invasive glucometer (OneTouch® Vita glucometer, LifeScan, France). Measures were repeated twice when values were out of normal range. Thyroxin ("T4," nmol.l^{-1}) was assayed in plasma samples (T4 ELISA kit, LDN®, ref TF E-2400) and the 6-point standard curve was adapted from 0 to 100 nmol.l^{-1}. Thiols (μmol.mgProtein^{-1}, SHp Test, Diacron Labs srl, ref MC432) and Glutathione Peroxidase ("GPx," U.mgProtein^{-1}, RANSEL, RANDOX, with controls, ref RS 504) were assessed from haemolysates of the red blood cells pellets. These last two parameters were normalized with protein concentrations using Bradford method (for detailed method, see Costantini et al., 2017).

Cellular Culture and Mitochondrial Analysis

Skin biopsies were washed in a series of PBS—70% alcohol—PBS baths and put in 50 μl 0.25% Trypsin solution (Gibco™, ThermoFisher scientific) for a 1-h digestion. Each sample was then transferred in a 96-well plate to form a fibroblastic cell line, and maintained in DMEM/F-12 medium (DMEM/F-12, GlutaMAX™ supplement, Gibco™, ThermoFisher scientific) mixed with 10% fetal bovine serum, 1% Penicillin-Streptomycin (Penicillin-Streptomycin (5,000 U.ml^{-1}), Gibco™) and 0.1% Amphotericin B (Gibco™ Amphotericin B 250 μg.ml^{-1}), in a 37°C incubator with 5% CO_2. Cells underwent 4 passages by adding 0.25% trypsin for 10 min, from the 96-well plate to 24-, 12-, 6-well plates and finally a T75 flask. They were then counted on a Malassez cell and 100 000 cells of each cell line were put in a Seahorse XF24 Microplate (Agilent Technologies) in triplicates. The protocol followed the instructions of the XF Cell Mito Stress Test Kit and analysis was performed using the Wave Software© (version 2.6.0.31, Agilent Technologies). The Oxidative Coupling Rate ("OxCR," % of baseline OCR) was calculated as the difference between the mean of the 3 initial measures of oxygen consumption rate ("baseline OCR," in pmole O_2.min^{-1}), and the minimum of the 3 measures following Oligomycin injection. Mitochondrial Reserve Capacity ("MtRC," % baseline OCR) was obtained by substraction of the mean of the 3 initial measures to the maximum of the 3 measures following FCCP injection ("stressed OCR" in pmoleO_2.min^{-1}). These parameters were expressed as a percentage of the baseline OCR in order to reduce inter-well variability due to cell number bias. The Glycolytic Potential ("GlcP," % of baseline ECAR) was extracted from ECAR measurements (in mpH.min^{-1}), as the difference between the mean value obtained after FCCP injection ("stressed ECAR," in mpH.min^{-1}) and baseline ECAR (mean of the first three measures), expressed as a percentage of baseline ECAR. It represents the capacity of the cells to use glycolysis in order to produce ATP (Divakaruni et al., 2014). Finally, the Metabolic Potential is the gap between OCR / ECAR in basal conditions ("basal" cell phenotype) and OCR / ECAR after FCCP injection ("Stressed" cell phenotype) and informs on the general capacity of the cells to meet an induced energy demand either by glycolysis or oxidative respiration (referred as the metabolic potential of the cells).

After each assay, cells were trypsinized from each well and pellets were collected after centrifugation. Cells were lysed in PBS buffer containing Proteinase K (0.2 mg.ml^{-1}), SDS (0.2%), EDTA (5 mM) for 3 h at 50°C. Total DNA was precipitated in 1.5 volume of cold ethanol with 0.2 M Sodium Acetate pH 5.2, and then centrifuged at 16,000 xg for 30 min at +4°C. After washing with 70% ethanol, pellet containing nuclear and mitochondrial DNA was resuspended in 10 mM Tris pH 8.0. DNA concentration was measured using fluorometry (Qubit™ 4 Fluorometer and Qubit™ dsDNA HS Assay Kit, Invitrogen™, ThermoFisher scientific). Mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) were quantified by qPCR using a 7300 Real-Time PCR System thermocycler (Applied Biosystems™). Primers for mtDNA (12S) and nuDNA (36B4) were designed from the gray mouse lemur genome as follows and ordered to Eurogentec: 12S-R: 5′-TAGCAAGAGGGGGTGAGGTT-3′; 12S-F: 5′-CCACGACAGCCAAGATCCAA-3′; 36B4-R: 5′-CCCATTCTATCATCAATGGGTACAA-3′; 36B4-F: 5′-CAGCAAGTGGGAAGGTGTAATCA-3′. We used Power SYBR® Green MasterMix (Applied Biosystem™, ThermoFisher scientific) as reaction media. The first thermic cycle was set to 95°C for 10 min, followed by 34 cycles of 95°C (30 s)−59°C (1 min)−72°C (1 min), then one final cycle of 95°C (1 min)-−55°C (30 s)−95°C (30 s). Amplification was measured at the last step of stage 2. Each sample was measured in two-time repeated triplicates and the mean value was used as data. The mtDNA/nuDNA ratio was used to account for intracellular contents in mitochondria.

Statistical Analyses

Results shown are given as means ± standard deviation (s.d) in tables and as least square means (LSM) with 95% confidence intervals in Figures, except for T4 and GlcP whose modelization was too complex to be identifiable. All parameters were measured for the 12 animals at each regimen ($N = 12$), except for mitochondrial analysis due to contamination during cellular culture ($N = 10$ for CTL, $N = 8$ for CR regimen). No outlier was identified or removed from data sets after testing with Dixon's Q test. Statistical analysis was conducted by the use of R software v 3.5.1 (R Core Team, 2016), and tests were considered significant when p-values were below the significant level set at 0.05. We applied linear mixed models with random individual effect on parameters with normal distribution (see **Table 2**) to test the effect of sex, caloric restriction and their interaction. Body mass was included in models only when its effect on the explained variable was significant. Age had a significant effect on 8-OHdG only, and was thus included in the model. For non-Gaussian parameters, generalized mixed models with random effects were used when supported by the data sets (used family is written in **Table 2**). When not supported, non-parametric pairwise Wilcoxon test was used as a default analysis, as shown in **Table 1**, which gathered *post-hoc* analysis (pairwise non-parametric tests for caloric restriction effect, unpaired for sex or interaction effect). Pairwise correlations were performed using pooled data collected before and after CR for each parameter of each sex to better describe possible links between variables, and were represented into a network graphic using the "corrr" 3.5.3 package (Ruiz et al., 2019). Principal component analyses (either with females and males together, or by sex) were performed using the "FactominR" 1.34 package (Le et al., 2008), and missing values imputed with the "missMDA" 1.11 package (Josse and Husson, 2016).

RESULTS

Caloric Restriction Induced a Stress Response in Both Females and Males

CR had a significant impact on cortisol production in both sexes (**Figure 1A**), as the plasma concentration of cortisol increased drastically after treatment (Chisq= 299, $p < 0.001$), with a higher response in females than males (+87.3 ± 142.4% vs. +43.5 ± 34.6%, Chisq= 62, $p < 0.001$; **Table 2**). One of the first expected effects of CR is the loss of body mass (BM). Although females began the experiment with a higher BM than males (99.7 ± 3.8 g vs. 90.0 ± 6.6 g, $p < 0.05$; **Figure 1B**), CR induced similar BM loss in both females and males (−16.7 ± 4.3% of the initial BM for females and −14.2 ± 4.3% of the initial BM for males, $p > 0.05$, **Table 1**) though taking into account the significant effect of the initial BM on BM loss ($p < 0.01$). Concomitantly with BM levels, females maintained a higher glycaemia than males during the experiment under control (95 ± 32 mg.dL^{-1} in females and 65 ± 9 mg.dL^{-1} in males, $p < 0.001$) and CR (84 ± 18 mg.dL^{-1} in females and 72 ± 11 mg.dL^{-1} in males, $p > 0.05$) conditions. However, the effect of CR on glycaemia differed as a function of sex (**Figure 1C**, $p < 0.05$; **Table 2**), with no change or very modest decrease in females (−1 ± 51% of the initial glycaemia) and a mean increase in males (+12 ± 26%).

CR Did Not Induce the Same Metabolic Adjustments in Males and Females

Mean oxygen consumption (VO_2, ml.kg.h^{-1}) was reduced after CR in males only, during both active (night: −10.2 ± 9.6%) and resting (day: −9.4 ± 8.8%) periods (**Figures 2A,C**, **3A**; **Table 2**). Conversely, VO_2 was increased after CR in females (+7.7 ± 15.9% during night and +9.6 ± 14.9% during day), this being

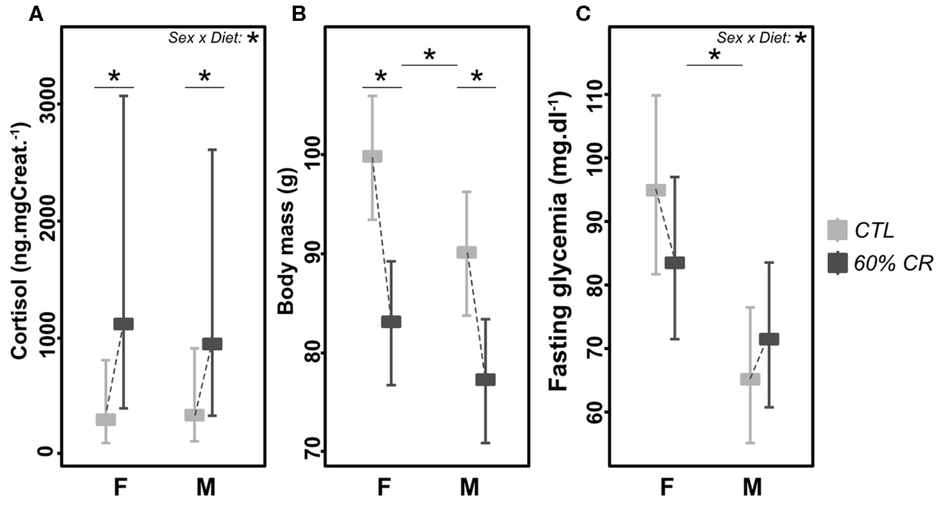


FIGURE 1 | Levels of **(A)** cortisol (ng*mg Creat.$^{-1}$), **(B)** body mass (g), and **(C)** fasting glycaemia (md*dl^{-1}) exhibited by female **(F)** and male **(M)** mouse lemurs before (CTL, light gray) and after (60% CR, dark gray) a 2-weeks exposure to a 60% caloric restriction. Data are given as least square means with 95% confidence intervals. Differences within sex groups (effect of CR, lower bars) and between sex groups (effect of sex, upper bars) are represented, as well as the significance of the interaction of Sex*Diet (CTL or CR). *< 0.05.

TABLE 1 | Mean and standard deviation of measured parameters for females (F) and males (M) in control treatment (ad libitum) and under 60% caloric restriction.

			Control		Initial Sex effect	60% Caloric restriction		CR effect		Sex effect in the caloric restriction response		
			F	***M***	***p-value***	***F***	***M***	***p-value F***	***p-value M***	***V°f (%)***	***V°m (%)***	***p-value***
Cortisol (ng.mgCreat.$^{-1}$)			603.4 ± 317.8	403.2 ± 111.5	-	992.3 ± 604.3	596.4 ± 245.3	-	°	+87.3 ± 142.4	+43.5 ± 34.6	-
8-OHdG (ng.mgCreat. $^{-1}$)			387.4 ± 93.0	591.0 ± 262.4	°	214.1 ± 36.3	324.6 ± 110.8	*	*	−42.4 ± 14.9	−42.7 ± 12.8	-
Thiols (μmol.mgProt. $^{-1}$)			1.8 ± 0.5	2.2 ± 0.3	-	1.8 ± 0.6	1.4 ± 0.2	-	°	+4.9 ± 187.1	−29.8 ± 44.8	°
GPx (U.mgProt. $^{-1}$)			0.28 ± 0.10	0.25 ± 0.17	-	0.34 ± 0.06	0.14 ± 0.04	-	-	+28.85 ± 49.06	−41.08 ± 19.71	-
T4 (nmol.l^{-1})			30.6 ± 8.5	32.4 ± 3.2	-	25.8 ± 8.8	21.3 ± 10.0	-	*	−6.4 ± 46.6	−35.6 ± 27.4	-
17 beta-Estradiol (pg.mgCreat. $^{-1}$)			33558.8 ± 15819.7	23364.4 ± 8297.4	-	60086.8 ± 13681.9	40180.7 ± 14563.2	*	*	+119.9 ± 102.9	+74.4 ± 35.6	-
Testosterone (ng.mgCreat. $^{-1}$)			2.8 ± 1.9	64.1 ± 31.1	***	12.9 ± 16.7	94.4 ± 78.0	°	–	+667.1 ± 1023.8	+32.8 ± 65.7	*
Glycaemia (mg.dl^{-1})			95 ± 32	65 ± 9	*	84 ± 18	72 ± 11	-	-	−1 ± 51	+12 ± 26	°
Body mass (g)			99.7 ± 3.8	90.0 ± 6.6	*	83.0 ± 5.1	77.2 ± 5.4	*	*	−16.7 ± 4.3	−14.2 ± 4.3	°
Oxidative coupling rate (% of baseline OCR)			60.96 ± 6.6	72.59 ± 8.3	°	69.49 ± 2.3	67.54 ± 2.1	-	-	+9.77 ± 7.78	−2.97 ± 12.06	-
Mitochondrial reserve capacity (% of baseline OCR)			202.4 ± 45.9	152.0 ± 21.8	°	148.9 ± 33.0	134.8 ± 41.8	-	-	−23.63 ± 24.07	−19.7 ± 18.51	-
Glycolytic potential (% of baseline ECAR)			325.28 ± 27.37	363.92 ± 203.20	-	407.43 ± 128.65	439.77 ± 35.41	–	–	+35.34 ± 36.25	+44.70 ± 101.04	–
Mt/Nu DNA Ratio			1.78 ± 0.43	3.01 ± 0.44	*	2.56 ± 1.16	3.30 ± 1.43	-	-	+2.99 ± 25.79	+60.06 ± 30.17	°
RER	NIGHT	Max	1.01 ± 0.04	0.97 ± 0.07	-	0.91 ± 0.04	0.95 ± 0.02	*	-	−9.50 ± 4.49	−0.81 ± 6.78	*
		hMax	18:23 ± 1 h39 min	17:44 ± 1 h 34	-	17 h 30 ± 41 min	17 h 17 ± 41 min	-	-	−50 min ± 1 h34 min	−27 min ± 1 h52 min	-
		Mean	0.99 ± 0.077	0.96 ± 0.099	-	0.85 ± 0.076	0.90 ± 0.085	*	°	−13.0 ± 3.7	−4.9 ± 5.9	*
	DAY	Min	0.74 ± 0.05	0.73 ± 0.02	-	0.75 ± 0.02	0.71 ± 0.06	-	-	1.24 ± 7.07	−3.19 ± 7.01	-
		hMin	07:00 ± 03 h 45 min	10:43 ± 3 h 12 min	-	03:53 ± 4 h 35 min	4 h 58 ± 3 h 58 min	-	°	−3 h 08 min ± 5 h 08 min	−5 h45 ± 4 h 28 min	-
		Mean	0.77 ± 0.07	0.78 ± 0.07	-	0.76 ± 0.06	0.75 ± 0.07	-	*	−0.9 ± 5.2	−3.6 ± 1.8	-
VO$_2$ (ml.kg^{-1}.h^{-1})	NIGHT	Max	2,343 ± 124	2,287 ± 266	-	2,301 ± 194	2,228 ± 344	-	-	−1.7 ± 8.3	−1.8 ± 17.7	-
		hMax	19:36 ± 3 h 04 min	19:01 ± 3 h 07 min	-	18:55 ± 3 h 56 min	17:39 ± 2 h 13 min	-	°	−41 min ± 2 h 19 min	−1 h 22 min ± 2 h 15 min	-
		Mean	2,147 ± 403	2,192 ± 510	-	2,260 ± 432	1,882 ± 473	-	*	+7.7 ± 15.9	−10.2 ± 9.6	°
	DAY	Min	1,075 ± 135	1,183 ± 162	-	1,162 ± 198	758 ± 233	-	*	+8.2 ± 14.5	−35.6 ± 18.4	**
		hMin	07:59 ± 2 h 09 min	06:58 ± 4 h 32 min	-	05:50 ± 3 h 34	04:05 ± 1 h 08 min	-	-	−2 h 19 min ± 5 h 25 min	−2 h 53 min ± 4 h 42 min	-
		Mean	1226 ± 406	1296 ± 425	-	1328 ± 598	1001 ± 382	-	*	+9.6 ± 14.9	−9.4 ± 8.8	*
EE (kcal.h^{-1})	NIGHT	Max	1.18 ± 0.09	1.04 ± 0.17	-	1.06 ± 0.22	0.85 ± 0.12	-	*	−9.2 ± 32.6	−17.9 ± 7.8	-
		hMax	19:59 ± 3 h 01 min	20:27 ± 3 h 54	°	17:58 ± 1 h 38 min	18:08 ± 4 h	-	-	−2 h 02 min ± 1 h 58 min	−2 h 20 min ± 3 h 39 min	-
		Mean	1.06 ± 0.15	0.92 ± 0.16	°	0.93 ± 0.11	0.71 ± 0.06	°	*	−11.7 ± 10.8	−21.2 ± 11.6	-
	DAY	Min	0.50 ± 0.05	0.49 ± 0.07	-	0.45 ± 0.07	0.28 ± 0.09	-	*	−9.5 ± 16.1	−41.8 ± 18.4	*
		hMin	08:53 ± 28 min	08:15 ± 4 h 45 min	-	07:07 ± 4 h 03 min	04:07 ± 1 h	°	-	−1 h 45 min ± 4 h 11 min	−4 h 08 min ± 4 h 55 min	-
		Mean	0.53 ± 0.06	0.52 ± 0.07	-	0.49 ± 0.09	0.41 ± 0.05	-	*	−6.7 ± 11.7	−20.1 ± 8.5	°

*The variation of parameters is calculated as the difference between restricted and control values. Non parametric tests were performed with or without pairing (Wilcoxon and Mann-Whitney, respectively). °<0.1, *< 0.05, **< 0.01, ***< 0.001.*

TABLE 2 | Analysis of deviance table (type II Wald Chi-square test) (***< 0.001; ** < 0.01; *<0.05; °< 0.1).

Parameter			**Body mass**		**Sex**		**Caloric restriction**		**Interaction**	
			Chisq	***p-value***	***Chisq***	***p-value***	***Chisq***	***p-value***	***Chisq***	***p-value***
Creatinine (mg.ml^{-1})			3.56	°	0.009	-	19.96	***	0.002	-
Cortisol (ng.mgCreat. $^{-1}$) *(poisson)*			153.71	***	0.07	-	299.47	***	61.78	***
8-OHdG (ng.mgCreat. $^{-1}$)			NS		13.26	***	743.64	***	0.01	-
(poisson)			Age effect : Chisq =7.41(**)							
Thiols (μmol.mgProt. $^{-1}$)			NS		0.55	-	4,01	*	6,69	**
GPx (U.mgProt. $^{-1}$)			NS		2.76	°	0.02	-	7.80	**
17beta-Estradiol (pg.mgCreat. $^{-1}$)			NS		5.11	*	30.28	***	1.52	-
Testosterone (ng.mgCreat. $^{-1}$) *(poisson)*			9.63	**	10.71	**	0.99	-	18.23	***
Glycaemia (mg.dl^{-1}) *(poisson)*			NS		12.54	***	0.51	-	5.69	*
Body Mass (g)					7.55	**	140.24	***	2.37	-
Body Mass loss (g) *(lm)*			Initial body mass		0.052		-			
			F-value= 10.9 **							
Oxydative coupling rate (mlO$_2$.min^{-1})			NS		3.83	°	0.43	-	6.23	*
Mitochondrial reserve capacity (mlO$_2$.min^{-1})			NS		2.61	°	5.57	*	1.29	-
Mt/Nu DNA Ratio			NS		4.03	*	2,86	°	0.08	-
RER	*NIGHT*	Max	NS		0.01	-	11.10	***	6.77	**
		hMax	9.40		0.37	-	0.55	-	0.62	-
		Mean	NS		0.70	-	38.34	***	7.40	**
	DAY	Min	NS		1.98	-	0.31	-	1.01	-
		hMin	NS		1.81	-	10.18	**	0.89	-
VO2 (ml.kg^{-1}.h^{-1})	*NIGHT*	Max	NS		0.34	-	0.33	-	0.01	-
		hMax	339.95	***	0.36	-	22.57	***	395.18	***
		Mean			3.13	°	0.42	-	5.25	*
	DAY	Min	NS		2.61	-	9.22	**	21.13	***
		hMin	NS		0.07	-	33.81	***	3.05	°
		Mean			0.003	-	0.013	-	7.37	**
EE (kcal.h^{-1})	*NIGHT*	Max	NS		6.57	*	7.02	**	0.36	-
		hMax	NS		0.02	-	9.02	**	334.48	***
		Mean	NS		8.28	**	20.07	***	1.01	-
	DAY	Min	NS		9.03	**	22.32	***	8.4	**
		hMin	NS		8.19	**	62.64	***	15.73	***
		Mean	NS		1.69	-	18.65	***	4.93	*

*Linear or generalized mixed effect models with random individual effect: Parameter ∼ Body mass + Diet*Sex + (1|Indiv). Body mass was implemented in models when applicable. For non-Gaussian parameters, generalized mixed models with random effects were used when supported by the data sets (family "poisson" mentioned when applicable). A simple linear model (lm) was built to test the effect of sex on body mass loss. Models did not converge for a few parameters (T4 and GlcP); for non-parametric analysis, see **Table 1**.*

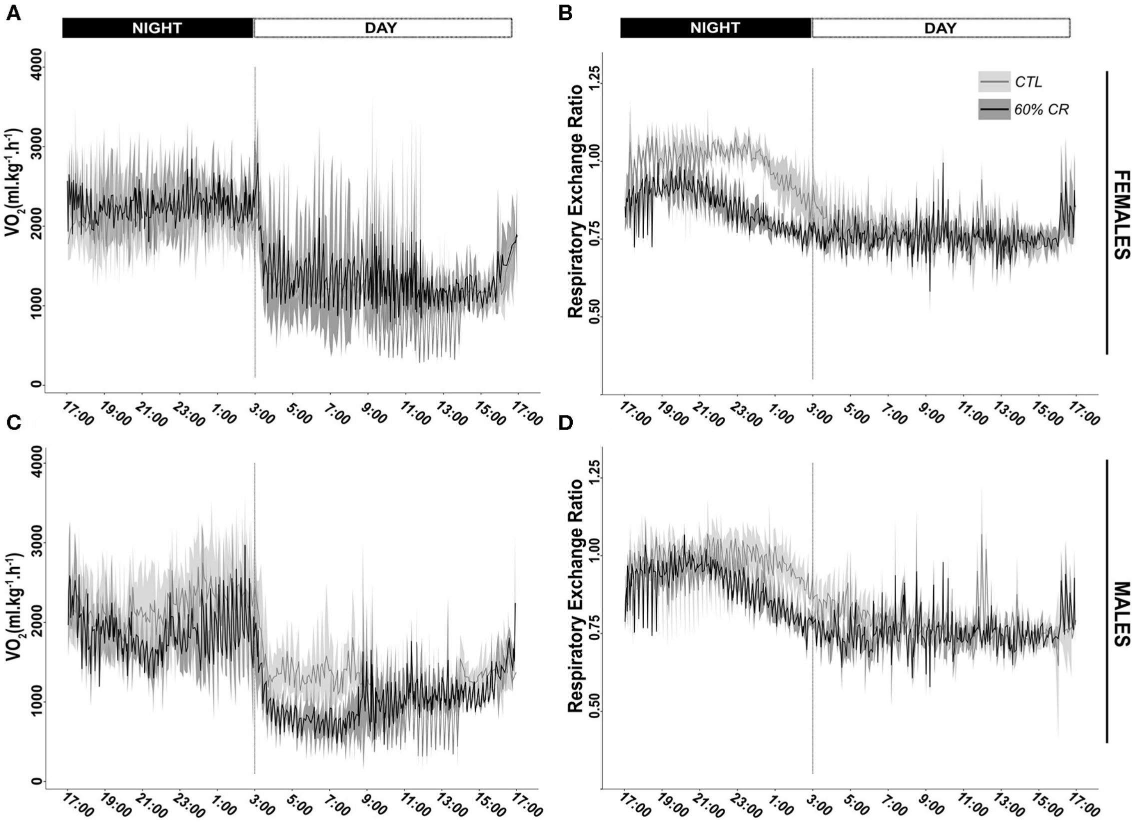


FIGURE 2 | Daily variations (mean ± sd) of oxygen consumption (VO$_2$ in ml*kg^{-1*}h^{-1}) and Respiratory Exchange Ratio (RER) measured in female (**A,B**, respectively) and male (**C,D**, respectively) mouse lemurs before (CTL, light gray) and after (60% CR, dark gray) a 2-weeks exposure to a 60% caloric restriction.

significant during the day only ($p < 0.05$; **Tables 1**, **2**). This sex-specific response to caloric restriction was accompanied by a modification of the temporal pattern of daily metabolic profiles (**Figure 4**). Indeed, the lower values of VO$_2$ were expressed earlier during the day after CR (i.e., resting phase) in both sexes (−2 h 19 min ± 5 h 25 min for females, −2 h 53 min ± 4 h 42 min for males), with an advanced time of maximum VO$_2$ during the night (i.e., active phase) (−41 min ± 2 h 19 min for females, −1 h 22 min ± 2 h 15 min for males) that was significantly sex-specific ($p < 0.001$; **Table 2**).

During the night in CTL situation (**Figures 2B,D**, **3B**), mean values of RER were close to 1 for both males and females (0.99 ± 0.08 for females and 0.96 ± 0.1 for males), reflecting their feeding behavior and indicating their nocturnal carbohydrate-depending metabolism. Under caloric restriction, mean nocturnal RER was decreased in both males and females (0.85 ± 0.076 for females vs. 0.90 ± 0.085 for males) indicating that animals relied less on carbs after feeding under CR as compared to the CTL situation. The daily profiles of RER (**Figure 2B**) clearly showed that animals had to switch from carb use to lipid oxidation earlier during the night under CR. Even though both sexes followed the same trend, the decrease in nocturnal mean RER was greater in females than in males (−13.0 ± 3.7% for females and −4.9 ± 5.9% for males, $p < 0.05$). However, males but not females showed a significant decrease in their mean RER during the day (−3.6 ± 1.8%), while females' response to CR was very heterogeneous (−0.9 ± 5.2%), which led to a significant sex*Diet effect ($p < 0.01$). The time of minimum and maximum expressions of RER during day and night (**Figures 4C,D** respectively), were significantly advanced after CR during the day ($p < 0.01$, **Table 2**), although it was not significant during the night and no sex effect could be pointed out (**Tables 1**, **2**).

As a product of VO$_2$ and RER, Energy Expenditure (EE, in kcal.hr^{-1}) was reduced in response to caloric restriction in both sexes during the night (−11.7 ± 10.8% for females and −21.2 ± 11.6% for males) when animals were active and fed themselves (**Figure 3C**). The nocturnal mean levels of EE were however higher in females than in males under both CTL (1.06 ± 0.15 kcal.hr^{-1} for females and 0.92 ± 0.16 kcal.hr^{-1} for males) and CR conditions (0.93 ± 0.11 kcal.hr^{-1} vs. 0.71 ± 0.06 kcal.hr^{-1} in females and males, respectively). During the day the mean energy expenditure was decreased by caloric restriction in all animals, but at a greater extent in males (−6.7 ± 11.7% for females and −20.1 ± 8.5% for males). This adjustment in EE levels was accompanied by a temporal shift in the daily profiles of energy expenditure, which was much more pronounced in males than females (**Figures 4E,F**). Indeed, the shift for an earlier energetic saving was much greater in males under caloric restriction (−1 h

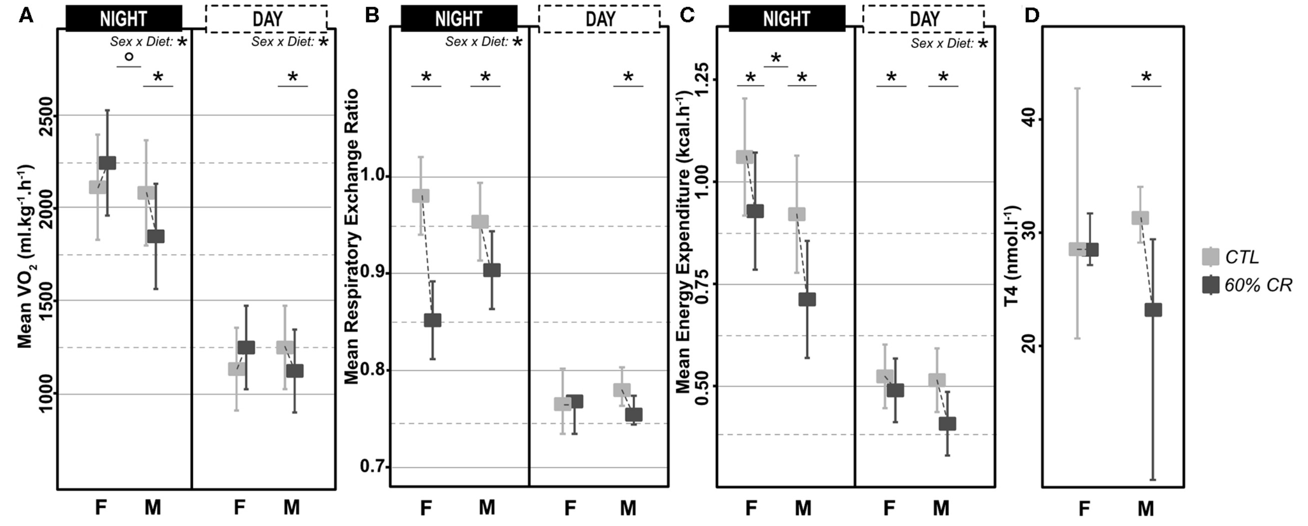


FIGURE 3 | Levels of **(A)** mean oxygen consumption (VO$_2$ in ml*kg^{-1*}h^{-1}), **(B)** Respiratory Exchange Ratio (RER), **(C)** mean Energy Expenditure (in kcal*h^{-1}) measured during the night (active phase) and the day (resting phase) in female (F) and male (M) mouse lemurs before (light gray) and after (dark gray) a 2-week exposure to a 60% caloric restriction. Thyroxinemia (T4, in nmol.l-1, **D**) was measured in the same animals during the resting period only. Boxes indicate least square means and error bars represent the 95% confidence interval, except for T4, which is represented as median and total range. Significant differences within sex groups (effect of CR, lower bars) and between sex groups (effect of sex, upper bars) are represented. $^* < 0.05$.

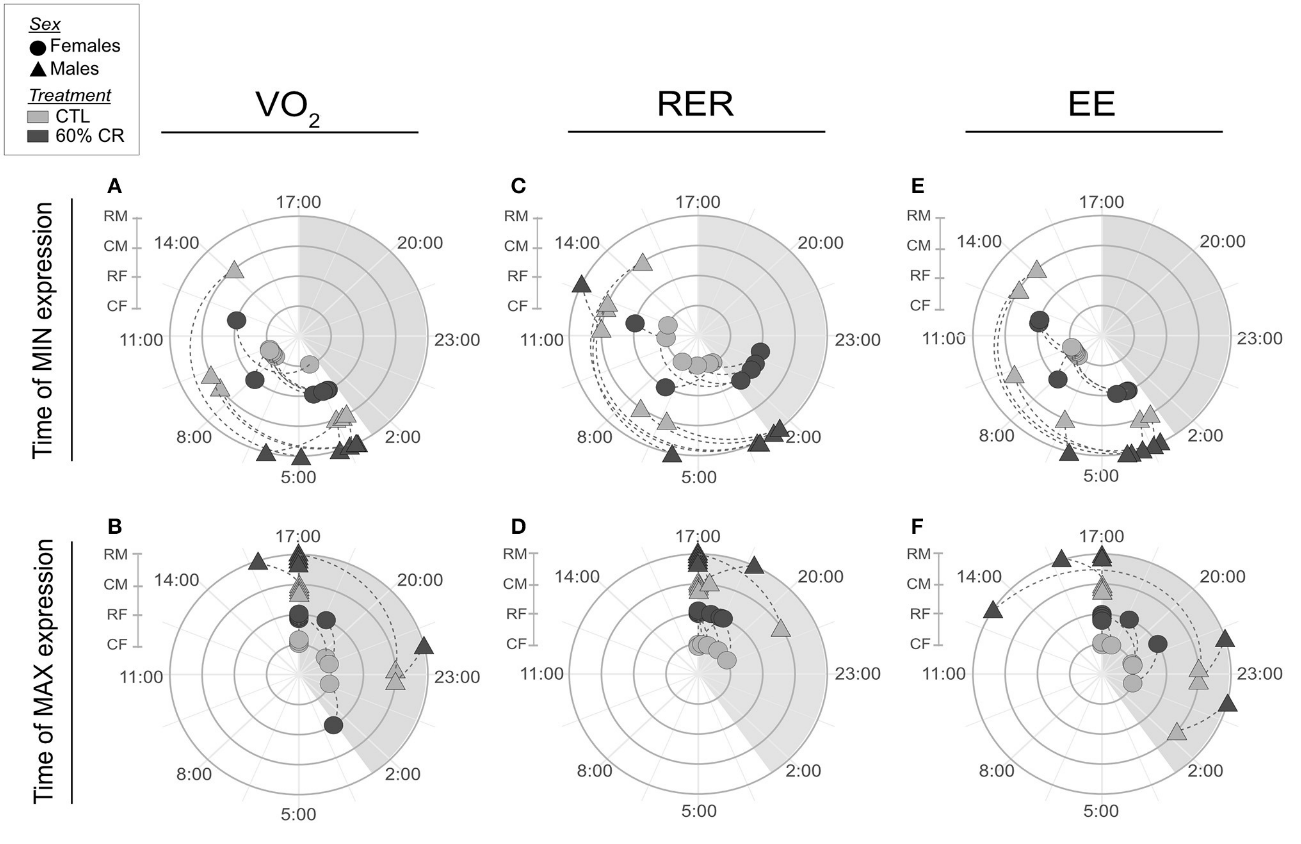


FIGURE 4 | Polar graphics representing averaged time (in hh:mm) when minimum and maximum VO$_2$ (**A,B**, respectively), RER **(C,D)**, and EE **(E,F)** were expressed over a 24 h period. Female mouse lemurs are shown as circles, and male as triangles. CTL animals are in ligth gray, CR in dark gray. Each experimental group is represented on its own concentric circle: RM (CR males), CM (CTL males), RF (CR females) and CF (CTL females). Time shift is represented by a dashed line for each individual. The shaded phase between 17:00 and 3:00 is the dark phase of the day, when the lights are off.

45 min ± 4 h 11 min for females vs. −4 h 08 min ± 4 h 55 min for males, $p < 0.001$; –). During the night, the level of maximum energy expenditure was also shifted due to CR, again at a greater extent in males (effect of sex in CR response $p < 0.001$; **Figure 4** and **Table 2**). Overall, males seem to respond more intensely and temporally to caloric restriction than females by decreasing their overall energy expenditure.

Thyroid hormones confirmed a sex-specific variation in metabolic activity due to caloric restriction (**Figure 3D**), with a significant decreased concentration of T4 in males only (−6.4 ± 46.6% of in females vs. −35.6 ± 27.4% in males; **Tables 1**, **2**).

Mitochondrial Activity Responds Differently to Caloric Restriction Between Sexes

Males' mitochondrial oxidative coupling rate (OxCR) measured from cultured fibroblasts was significantly higher than females' under control conditions (**Figure 5A**). OxCR's response to CR varied according to sex (sex*Diet, $p < 0.05$; **Table 2**), as it increased in females after caloric restriction (+9.77 ± 7.78% of baseline OCR), while a slight decrease was observed in males (−2.97± 12.06% of baseline OCR), thus leveling females' values to those of males' after CR. This rather opposite response of oxidative rate to CR between sexes did not prevent the decrease of mitochondrial reserve capacity (MtRC) in each case (**Figure 5B**), which was reduced at the same extent due to CR, although females began the experiment with a higher MtRC than males (202.4 ± 45.9% of baseline OCR vs. 152.0 ± 21.8% of baseline OCR, $p < 0.1$; **Table 1**). Glycolytic potentials ("GlcP," **Figure 5C**) increased under CR showing the enhanced capacities of the cells to use glycolysis under food restriction (+35.3 ± 36.3% of baseline ECAR for females and +44.7 ± 101.0% of baseline ECAR for males, $p < 0.05$; **Table 2**). From a qualitative assessment, the OCR/ECAR quotients clearly showed a shift from a quiescent phenotype at baseline to a more energetic phenotype after FCCP exposure in all experimental groups, not favoring either the glycolytic or oxidative pathway (**Figure 6**). Also, caloric restriction reduced the metabolic potential (defined as the difference between the baseline and the stressed condition) of both females and males, although this reduction seemed to be greater in females than in males. However, females consistently showed a greater potential than males, both before and after CR (**Figure 6**). Mt/Nu DNA ratios (**Figure 5D**) increased after CR in both sexes (+ 3 ± 26 in females, +60.1 ± 30 in males, $p < 0.1$; **Table 1**) but males had an overall higher ratio than females before and after CR ($p < 0.05$, **Table 2**).

CR Affected the Oxidative Status, Through Different Mechanisms in Males and Females

Oxidative DNA-damage (8-OHdG, **Figure 7A**) showed a higher overall concentration in males than females (387.4 ± 93.0 for females vs. 591.0 ± 262.4 ng.mgCreat.$^{-1}$ for males under CTL diet, $p < 0.1$; 214.1 ± 36.3 for females vs. 324.6 ± 110.8 ng.mgCreat.$^{-1}$ for males after CR), but was decreased after caloric restriction at the same extent in both sexes (−42.4 ± 14.9% for females and −42.7 ± 12.8% for males, $p > 0.1$). However, thiols (**Figure 7B**) and glutathione peroxidase (GPx, **Figure 7C**), both markers of anti-oxidant activity, followed the same trend in response to CR, though with opposite directions between females and males (significant interaction effect, $p < 0.01$ in both parameters). Indeed, CR induced an increase in thiols (+4.9 ± 187.1%, $p > 0.1$) and in GPx (+28.85 ± 49.06%, $p > 0.1$) in females, but a decrease in males (thiols: −29.8 ± 44.8%, $p < 0.1$; GPx: −48.08 ± 19.71%, $p > 0.1$).

Caloric Restriction-Induced Modulation of Sexual Hormones

Urinary estradiol was significantly higher in females as compared to males during the experiment (Chisq= 5.11, $p < 0.05$; **Figure 8A** and **Table 2**). However, CR induced an increase in both sexes (Chisq = 30.28, $p < 0.001$; **Table 2**). In parallel, testosterone was very low in females as compared to males (2.8 ± 1.9 ng.mgCreat.$^{-1}$ for females and 64.1 ± 31.1 ng.mgCreat.$^{-1}$ for males in CTL situation, **Figure 8B**), and exposure to CR induced a significantly increased concentration in females only (+677.1 ± 947.8% in females against +32.8 ± 62.6% in males, <0.05; **Table 1**), though female levels remained much lower than males' after CR.

Integrative Analyses Confirmed an Effect of Sex in Response to CR

General PCA analysis (**Figure 9**) showed a clear discrimination between the 4 experimental groups around the 2 first principal components (PCs), which explained ∼50% of the variation in the dataset. First, the analysis demonstrates that males and females started the experiment with different physiological states. PC1 explained about 31% of the variation and significantly discriminated CTL females ($p < 0.01$) and CR males ($p < 0.001$) from the rest of the individuals. The fattest animals (CTL females) were also the ones who had the biggest mitochondrial reserve capacity (MtRC) and the highest energy expenditure (EE) during the day. This was accompanied with a higher thyroxinemia and glycaemia than every other animal (**Figure 9B**). In contrast, CTL males were characterized along PC2 (which explained around 17% of variability) by their levels of oxidative damage, blood thiols and mitochondrial oxidative coupling rate (**Figure 9B**). Animals that had the highest levels of oxidative DNA damage (CTL males, $p < 0.001$) were the ones presenting the highest concentrations of thiols and also secreting the lowest cortisol. Caloric restriction induced contrasted effects between males and females along the 2 PCs. Thus, the effect of CR in females was mostly explained by parameters representative of mitochondrial respiration (GlcP, OxCR and the ratio Mt/Nu DNA) as well as estradiol and cortisol levels (**Figure S1B**), some of which (estradiol and cortisol) significantly contributed to discriminate the CR females from the other animals (**Figure 9**). Correlations networks (**Figure S2**) further confirmed strong differences between males and females in the relationship between metabolism and oxidative status. Indeed, energy-related parameters (energy expenditure, VO2, RER; thyroxin levels) were

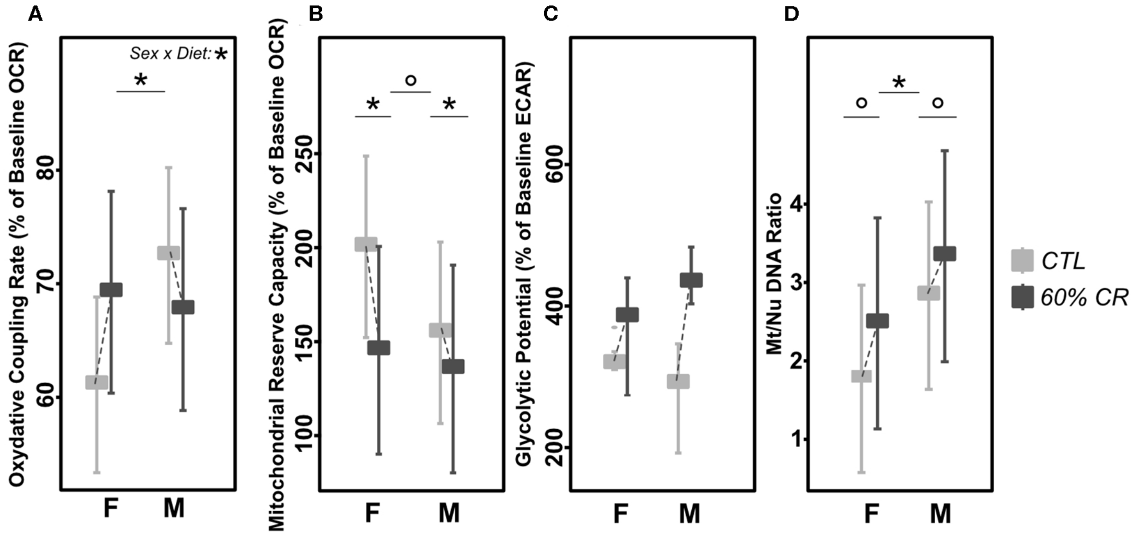


FIGURE 5 | Levels of **(A)** mitochondrial oxidative coupling rate (% of baseline OCR), **(B)** Mitochondrial reserve capacity (% of baseline OCR), **(C)** Glycolytic Potential (% of baseline ECAR), and **(D)** Mt/Nu DNA ratios measured in cultured fibroblasts from female (F) and male (M) mouse lemurs before (CTL, light gray) and after (60% CR, dark gray) a 2-week exposure to a 60% caloric restriction. Boxes indicate least square means and error bars represent the 95% confidence interval, except for GlcP (median and total range). Significant differences within sex groups (effect of CR, lower bars) and between sex groups (effect of sex, upper bars) are represented, as well as the significance of the interaction of Sex*Diet (CTL or CR). *< 0.05; ° < 0.1.

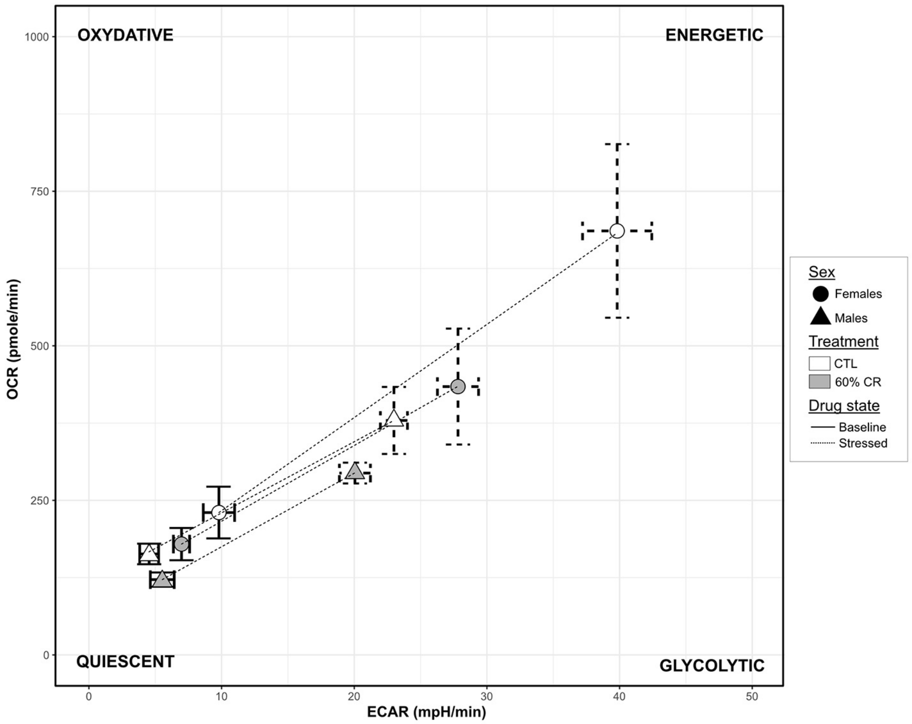


FIGURE 6 | Graphic representation of the cellular metabolic potential measured in cultured fibroblasts from female (circles) and male (triangles) mouse lemurs before (CTL, white) and after (60% CR, gray) a 2-week exposure to a 60% caloric restriction. Metabolic Potentials (mean + sd) are represented by dashed lines between two cell energy phenotypes: "Basal OCR/ECAR" (sd in solid lines) and "Stressed OCR/ECAR" (sd in dashed lines; determined under FCCP conditions, chemically mimicking an induced energy demand). The 2-dimension description of the metabolic potential informs on the general capacity of the cells to meet an energy demand either by glycolysis ("Glycolytic" phenotype), oxidative respiration ("Oxydative" phenotype) or both ("Energetic" phenotype).

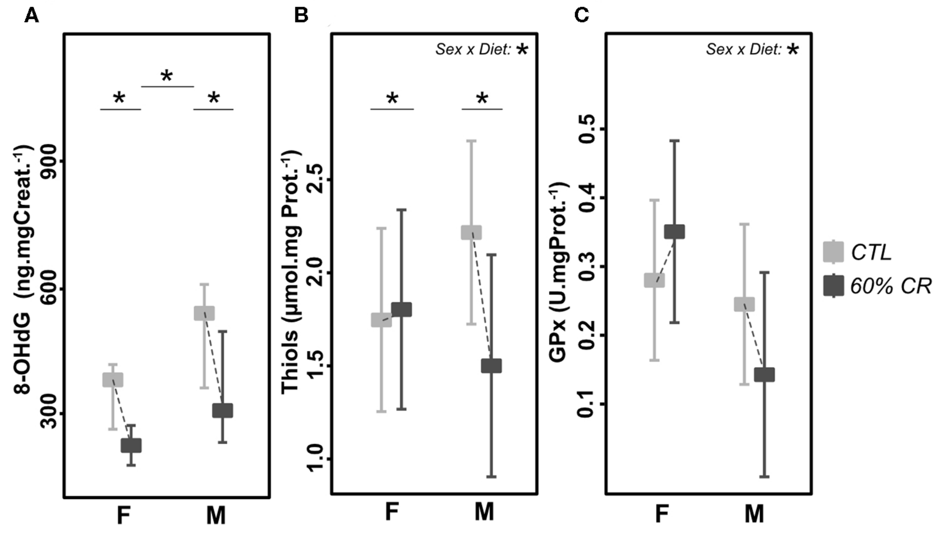


FIGURE 7 | Levels of **(A)** 8-OHdG (ng*mg Creat.$^{-1*}$g.$^{-1}$), **(B)** Thiols (µmol*mg Prot.$^{-1}$), and **(C)** Gluthatione Peroxydase (GPx, U*mg Prot.$^{-1}$) measured in female **(F)** and male **(M)** mouse lemurs before (CTL, light gray) and after (60% CR, dark gray) a 2-week exposure to a 60% caloric restriction. Boxes indicate least square means and error bars represent the 95% confidence interval, except for 8-OHdG (median and total range). Significant differences within sex groups (effect of CR, lower bars) and between sex groups (effect of sex, upper bars) are represented, as well as the significance of the interaction of Sex*Diet (CTL or CR). *< 0.05.

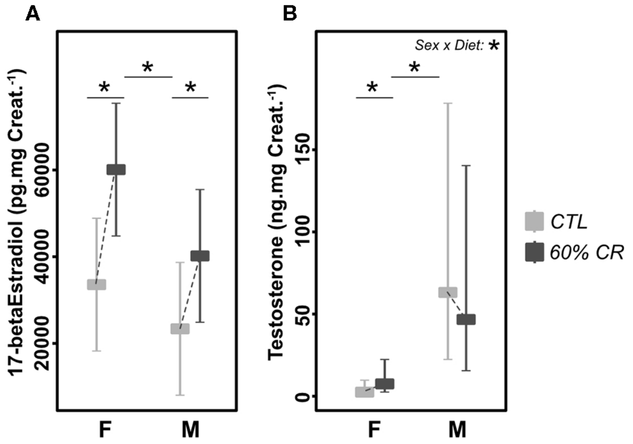


FIGURE 8 | Levels of **(A)** 17-beta Estradiol (pg.mg Creat.$^{-1}$) and **(B)** Testosterone (ng.mg Creat.$^{-1}$) measured in female **(F)** and male **(M)** mouse lemurs before (CTL, light gray) and after (60% CR, dark gray) a 2-week exposure to a 60% caloric restriction. Boxes indicate least square means and error bars represent the 95% confidence interval. Significant differences within sex groups (effect of CR, lower bars) and between sex groups (effect of sex, upper bars) are represented, as well as the significance of the interaction of Sex*Diet (CTL or CR). *< 0.05.

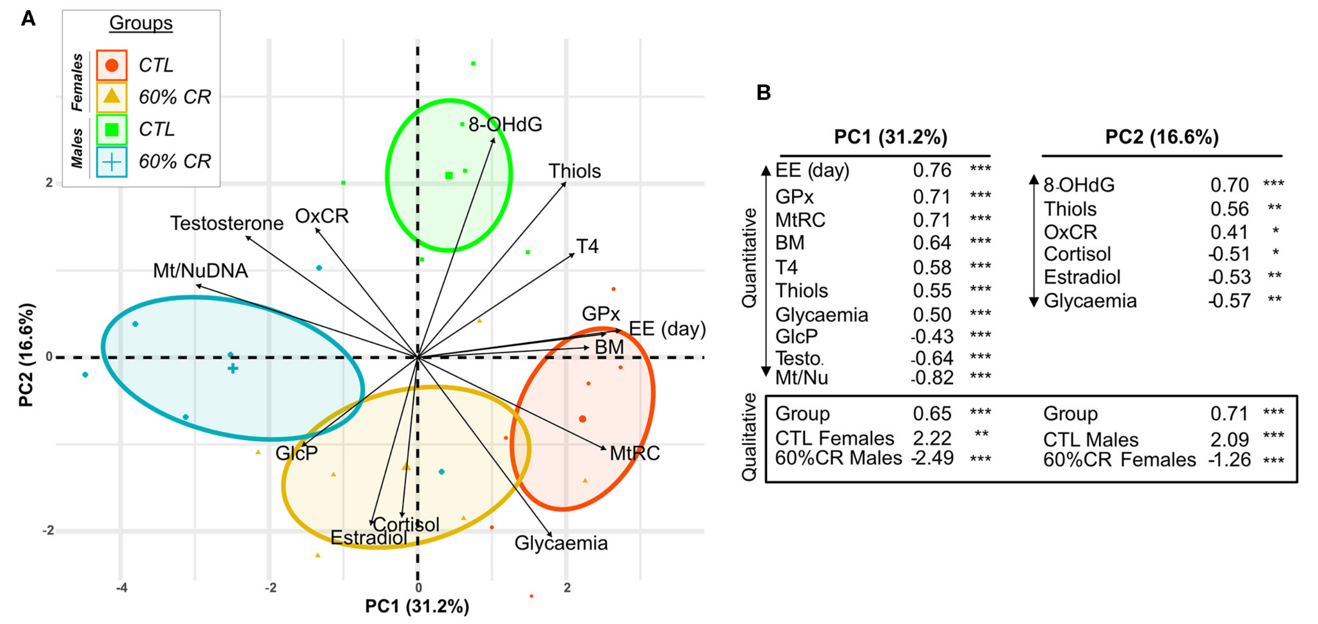
clearly positively correlated with oxidative damage and anti-oxidant activity in males. Such interaction was not evidenced in females, markers of anti-oxidant activity being even negatively correlated to energy expenditure before and after CR.

DISCUSSION

Facing food shortage after reproductive investment might be very challenging, though females and males might respond unequally to such unpredictable stress. We tackled this question by comparing the response of female and male mouse lemurs to a 2-weeks 60% caloric restriction (CR) applied at the end of the reproductive season. Our results confirmed that CR triggers a different response between males and females, characterized by sex-specific metabolic, anti-oxidant and mitochondrial regulations, which provides experimental evidence for the necessity to consider sex as a factor for population dynamics in climate change models.

Females Completed Their Summer-Like Season in Better Body Condition Than Males

At the end of summer, many different biological and behavioral events have specifically distinguished males and females, and this could introduce a bias when facing environmental perturbations. With the direct observation that the male sample in this study had an initial lower body mass (BM) compared to females, as it is also the case in Brunoy's breeding colony during this time of the life cycle, we kept in mind that initial BM could influence each sex response to CR differently. In this respect, initial BM was always included in the statistical models and was either left or removed in case of significant or non-significant weight, respectively. However, the mean initial BM in each group was far from representing the poor body condition or "exhaustion" that we could expect to find in wild populations (as animals from both sexes have been found to weight up to ~60 g during the transition to winter, Schmid and Kappeler, 1998). Also, the sex-gap of this particular parameter, although statistically significant, is very limited compared to the extreme BM variations one individual can display throughout the year (with an amplitude of ~50% of the mean BM variation over 1 year, Perret et al., 1998). Nevertheless, even after spending



	PC1 (31.2%)			PC2 (16.6%)		
Quantitative	EE (day)	0.76	***	8-OHdG	0.70	***
	GPx	0.71	***	Thiols	0.56	**
	MtRC	0.71	***	OxCR	0.41	*
	BM	0.64	***	Cortisol	-0.51	*
	T4	0.58	***	Estradiol	-0.53	**
	Thiols	0.55	***	Glycaemia	-0.57	**
	Glycaemia	0.50	***			
	GlcP	-0.43	***			
	Testo.	-0.64	***			
	Mt/Nu	-0.82	***			
Qualitative	Group	0.65	***	Group	0.71	***
	CTL Females	2.22	**	CTL Males	2.09	***
	60%CR Males	-2.49	***	60%CR Females	-1.26	***

FIGURE 9 | Principal Component Analysis discriminating female and male mouse lemurs before (CTL, red and green for females and males, respectively) and after (60% CR, orange and blue for females and males, respectively) a 2-week exposure to a 60% caloric restriction. Parameters indicative of energy balance (Body mass "BM"), metabolic activity (Cortisol "Cortisol," Thyroxin "T4," Mean EE over day "EE (day)," mitochondrial respiration (Oxydative coupling rate "OxCR", Mitochondrial Reserve Capacity "MtRC," Glycolytic Potential "GlcP"), Oxidative status (8-OHdG "8-OHdG," Thiols "Thiols," Glutathione Peroxydase "GPx"), sexual hormones (Estradiol "Estradiol," Testosterone "Testosterone") were included in the analysis. Individual plots are shown in **(A)** and grouped into sex*Diet. The contribution of each parameter is also represented by black arrows displaying the "Variable factor map." Principal Component 1 (PC1) indicates ∼31% of variability and Principal Component 2 (PC2) ∼17%. **(B)** Table of correlation coefficients and corresponding *p*-values shows only the variables significantly contributing to the two first principal components of the analysis (Quantitative) and the discrimination of qualitative variables: CTL Females, 60%CR Females, CTL Males, 60%CR Males. *< 0.05, **< 0.01, ***< 0.001.

summer under the "favorable" conditions of Brunoy's colony, with regular and predictable food intake, our results confirmed that CR triggers different mechanisms between males and females at the end of the summer-like period, characterized by sex-specific metabolic, anti-oxidant and mitochondrial properties. In this line, results from multivariate analyses confirmed that BM was not the primary contributor of the sexual differences observed in response to CR.

Caloric Restriction Was Perceived as a Stressor During the Summer-Like Period

One remarkable result was the significant and consistent cortisol increase in urine samples after CR in both females and males, which could point out a stressed physiological status (for review, see Breuner et al., 2013). The effect of CR on cortisol concentrations seems to depend on its intensity and duration; in a recent meta-analysis, only fasting (from 2.5 to 6 days) was responsible for an increase in cortisol in human patients, while a 60% CR between 1 to 28 days did not (Nakamura et al., 2016). Previous work in rats showed a dose-dependent response of the increase in serum corticosterone after 3 weeks of CR (Levay et al., 2010). In mouse lemurs, a 60% CR applied to 24 females in winter-like period, as well as a chronic food shortage of 40% followed by a 80% acute CR in 6 individuals induced no urinary cortisol effect as well (Canale and Henry, 2011). The present experimental study was actually the first to test the effect of CR at this advanced time of the summer season, which could explain the difference with the results mentioned above. We found that a 60% CR undergone after the reproductive effort is therefore felt as a stress for these highly flexible primates, thus confirming the unexpected nature of such food shortage during a usually well supplied season (Dammhahn and Kappeler, 2008). In contrast, mouse lemurs are physiologically well-adapted to food shortage during winter (a dry period with low food availability), which thus triggers low stress response but induce hypometabolism states instead (Giroud et al., 2008; Vuarin et al., 2015).

Males and Females Show a Similar Energy Balance but Different Metabolic Responses to CR

As lemurs went through their energetic challenges of the year related to reproduction—though these challenges seem to be quite moderated for females in captive conditions (Landes et al., 2019)—late summer is considered as a period of nutritive abundance and possibly fattening in healthy natural populations that anticipate the poor season (Radespiel, 2006; Radespiel et al., 2006; Dammhahn and Kappeler, 2008). At the time of the experiment, males had undergone their reproductive investment and were sexually inactive, although testosterone levels were still high before and after CR. Females had also completed gestation and parental care, and did not exhibit late estrus. Therefore, we considered that male and female mouse lemurs probably shared equivalent energy allocations, although the experimental design did not allow to firmly rule out any confounding factor due to the natural modulations in metabolic activity that might have happened within the 2-weeks duration of the experiment, as animals were their own controls. Nevertheless, mouse lemurs are supposedly stable in their metabolic and reproductive status at this time of the year in captive conditions (Perret and Aujard, 2001). In these conditions, males and females lost similar amount of weight following CR, in total and relative to their initial body mass. The latter had an impact on the amount of weight loss, and thus erased any sex effect in the individuals' reaction to caloric restriction. In the same time, the mitochondrial reserve capacity (MtRC) decreased in both sexes, reflecting the impact of CR on the animals' ability to face new environmental stressors, as the cell's capacity to meet a new energy demand is drastically reduced under impaired caloric intake. Furthermore, MtRC was indeed positively correlated with body mass (**Figure 9**, **Figures S1**, **S2**), which strengthens its interpretation as an energy-balance marker.

However, the underlying metabolic indicators presented high variability between sexes, with an overall diminution in males (decrease in night and day oxygen consumption—VO_2, Energy Expenditure—EE, Thyroxin parameters, no change for Oxidative Coupling Rate—OxCR) but very contrasted changes in females (mean VO_2 increased during the day and did not vary with CR during the night, resulting in an unchanged EE during the day and decrease over night; no effect of CR was observed on T4 but OxCR increased). We can thus argue that even if CR was perceived as a stressor by both sexes, and induced similar energy balance, there is conclusively a sex-specific physiological response to this type of stressor at the end of summer. These results suggest that males entered a hypometabolic state, while females did not, particularly during the day when torpor, which is an energy-saving mechanism (Vuarin et al., 2015), occurs. As we used the total body mass to normalize VO_2 measurements instead of the lean body mass (LBM), which is supposed to be the metabolically active portion of the total weight, the male-female VO_2 gap after CR is likely to be underestimated. Moreover, in regard to the final energy balance, hypometabolism was not efficient in males and was not followed by a decrease of ATP needs, as OxCR informs on the energy production rates of the cells (Divakaruni et al., 2014). In contrast, females' ATP demand increased in response to CR, but was still significantly lower than in males. Aside from this, we observed an increase in glycolysis potentials (GlcP) after CR in both sexes, meaning that the cells were able to better use glycolysis in case of an extra fuel need under stressed conditions and maximal mitochondrial activity. CR seemed to favor a more direct type of response to environmental stress with increased glycolysis capacities, while MtRC and thus maximal mitochondrial respiratory capacity under stress lowered drastically and could not provide full proficiency. Cellular metabolic potential was decreased after CR showing an altered response toward induced cellular energy demand, but were still—although qualitatively assessed—higher in females. For these cellular-scaled parameters we can still argue on the link between metabolism and fibroblasts, whose response may not represent the one of cells more implied in the regulation of metabolic activity or "metabolic tissues" (as hepatocytes, adipocytes, pancreatic cells or myocytes) (Patel et al., 2014). However, fibroblasts are constitutive of the skin, the barrier between the internal and external environment of an organism, that is capable of integrating many signals which *in fine* impact homeostasis (for review see Slominski et al., 2013). As such, even if fibroblasts are not key actors in the regulation of metabolism, they can still respond to an organism's regulation, and they are known to be up and down-regulated along with key metabolic pathways, as glycolysis and fatty acid oxidation (Zhao et al., 2019). Our method showed good discrimination of mitochondrial activity under CR, sparing the necessity to perform more invasive sampling.

Observations in the little brown bat (*Myotis lucifugus*) converged toward the "thrifty female hypothesis" (TFH; Jonasson and Willis, 2011), in which females display a greater resistance to weight loss than males, as they supposedly experience greater energetic challenges after the dry season, when they face spring reproduction (Key and Ross, 1999). This selection pressure would have driven females to be more efficient in using energy saving mechanisms, or "thrifty phenotypes" (TP), such as torpor, hibernation or fat storage (CZenze et al., 2017; Willis, 2017). The TFH has been originally enunciated to explain sex-variability in the expression of these TP during winter (Humphries et al., 2003; Jonasson and Willis, 2011), but no study has yet explored its generality throughout the year. Indeed, if the expression of a TP in females is dependent on their energy allocation to reproduction, as stated in the TFH, it is worth considering the case of a more constitutive phenomenon, where females' TP could also be expressed after reproductive investment. There is actually growing evidence that females of different species could present physiological abilities compared to males in their response to caloric restriction and energy storage independent of or during gestation (Rodriguez-Cuenca et al., 2002; Valle et al., 2007; Lennox and Goodship, 2008; CZenze et al., 2017). Overall, in the present study, the comparison of physiological responses to CR between males and females did not support the hypothesis that female lemurs might express a more "thrifty" phenotype in late summer, as the energy balance was similar between sexes, and there was a reduced metabolic activity in males. We cannot rule out the fact that females were heavier than males before and after the experiment which might have influenced their response to CR. Indeed, body condition is known to interfere with the use of hypometabolism (Bieber et al., 2014). Given that hypometabolism is associated with adverse effects such as accumulation of molecular oxidative damages (Wei et al., 2018), the "decision" not to initiate it might be more adaptive in females at that season time, according to the fact that they had sufficient reserves to undergo the cost of CR. Whether females would have modified their response to CR with a prolonged stress remains an open question.

Sex-Specific Oxidative Status Management in Caloric Restriction Conditions

Despite CR was perceived as a stress in regard to the individuals' cortisol increase, urinary oxidative damage to DNA (8-OHdG) decreased in both sexes. It was initially lower in females (and remained lower after CR), which suggests an overall greater oxidative stress in male mouse lemurs at the end of summer. 8-OHdG positively correlated with metabolic activity parameters only in males (mean EE during the day, thyroxin—T4, OxCR see **Figure 9**, **Figures S1B**, **S2**) according to our expectations and the Free radical theory of aging (Harman, 1956). Indeed, intense oxidative metabolism produces reactive oxygen species (ROS) that damage molecular structures, potentially inducing cellular and tissue harm, which eventually contribute to senescence (for review, see Kregel and Zhang, 2007). Anti-oxidative machinery intervenes as a protective mechanism (Pamplona and Costantini, 2011) and could be regulated by metabolic rate as well. Here we observed contrasted relationships between metabolic rate, T4, 8-hydroxy-2'-deoxyguanosine−8-OHdG, glutathione peroxidase and Thiols in males and females mouse lemurs. Indeed, caloric restriction induced concomitant decrease in T4, 8-OHdG and antioxidant machinery in males, thus compelling with the "rate-of-living" theory. In females however no or little change in energy expenditure was observed after CR, which was corroborated by the absence of variation in T4 measurements, while thiols (and GPx to a lesser extent) significantly increased (yet very contrasted amongst the individuals) and 8-OHdG was still reduced, supposedly as a direct consequence of the anti-oxidant activity. Although metabolic activity could be directly implied to some extent in the resorption of oxidative damage (Pamplona and Costantini, 2011) as we observed in males, females seem to activate other pathways, such as the up-regulation of anti-oxidant mechanisms.

We do not know if the intersexual variability in the regulation of oxidative status is a direct effect of sex or is linked to the difference of metabolic rate response induced by CR. As it is discussed in many studies, oxidative stress could be modulated either by food shortage or sexual hormones, and the existence of a sex-specific oxidative balance is debated (Costantini, 2018). Caloric restriction has been robustly demonstrated to improve maximum life span in mammals, including primates (Masoro, 2005; Pifferi et al., 2018) and this effect was correlated with the reduction of oxidative damage accumulation in several experiments (for review, see Sohal and Weindruch, 1996). Recently the protective effect of chronic CR on oxidative stress was diminished in mice that lacked type-3 sirtuin SIRT3, a mitochondria-localized protein which promotes the anti-oxidant properties of type-2 superoxide dismutase SOD2 (Qiu et al., 2010), indicating a major upregulation of the antioxidant machinery during CR. This particular result is in line with our findings in females, but not in males (although the sex of mice was not specified, nor discussed in the above mentioned article). It is also pointed out that estrogen, in contrast to other natural steroids, possess an antioxidant activity because of the phenolic structure in the molecule, and this specificity was assessed *in vitro* in 1987 (Sugioka et al., 1987). Additionally, a positive correlation was observed between estrogen and GPx (Massafra et al., 1998), which supports the hypothesis of an estrogen-dependent regulation of the antioxidant machinery. In rats, castration decreased SOD activity in both sexes, although more intensely in females, and highly increased lipid peroxidation (in a model of myocardial damage) in females, but not in males (Barp et al., 2002). In healthy aged rats finally, females were proven to host lower mitochondrial content than the males (which was supported by our Mt/Nu DNA results), but with a greater differentiation degree and higher activity (Guevara et al., 2009). This was accompanied by a better "oxidative balance" in females, meaning higher respiratory function followed by equal ROS production, but greater antioxidant enzyme activity, and uncoupling proteins (Guevara et al., 2009). All these information converge to a sexual difference in the oxidative status regulation, also in a context of CR, where females are able to lower the damage induced by the perceived stress by upregulating their antioxidant machinery, while males respond by decreasing their metabolic activity, which also decreases ROS production.

As predicted from the above studies, estrogen increased in response to CR in females. The effect was also observed in males, which represents an original result as few studies gather information on the potential role of estrogen as regulator of the oxidative status in males. Our study also provides a first glimpse of *Microcebus murinus* males' estrogen concentrations. We did not find any estrogen-antioxidant correlation however to support the role of the sexual steroids in the regulation of oxidative status, but estradiol levels did correlate negatively with 8-OHdG (see **Figure 9**, **Figures S1**, **S2**).

On the other hand the anti-oxidant effect of testosterone remains unclear, as it has been shown to either depress red blood cell resistance to free radical attack in a male zebra finch model treated with testosterone (Alonso-Alvarez et al., 2007), or decrease ROS production and increase GPx activity and thiols groups content in an *in vitro* human neutrophil culture (Marin et al., 2010). Mouse lemur males expressed more than 20 times the testosterone concentrations of females, yet CR still had a significant impact on females' testosterone levels (**Figure 8**), while it remained unchanged in males. As testosterone can be metabolized into estradiol and dihydrotestosterone, their respective oxidative/antioxidant properties may be difficult to tease apart. This idea was explored in 2012 in birds (Casagrande et al., 2012), which showed an antioxidant activity of estradiol only in both sexes. The increase in female's testosterone may be due to the necessity of producing higher levels of estrogens, while the "stock" would be already available in males.

On Sex-Biased Ecological and Evolutionary Perspectives

The final energy balance in response to CR was similar between males and females in the present study. However, the metabolic rate and oxidative damage modulations were significantly different between sexes. Metabolic activity markers of females were not decreased after CR (T4, OxCR, VO_2) which could illustrate how physiology is adapted to the different biological and behavioral features of sexes in *Microcebus murinus* during this late-summer context, when females still take care of their offspring in nature and form kin-groups to endure winter (Radespiel et al., 2001). Both body mass and mitochondrial reserve capacity were lower in males at the beginning of the experiment (and remained as such afterwards) suggesting that summer would be a more challenging period for them, even in breeding conditions. This conclusion is also supported by the higher levels oxidative damage to DNA.

The major constraint for the fitness of males that could drive sexual selection and dimorphism is thought to be the limited access to females for mating, that would thus favor larger body size or other energetically costly physical features (Darwin, 1874; Emlen and Oring, 1977; Key and Ross, 1999). As opposed to this, females' reproductive success would be constrained –only-by food resources (Trivers, 1972). In case of a monomorphic species, females' energy expenses were demonstrated to exceed that of males, mainly because of gestation and lactation, which costs can be easily monitored (Key and Ross, 1999). The males' specific energy expenditure was actually calculated during the time it took for females to produce an offspring. This particular paper did not take into account the sex-dependent phenological shift of maximal reproductive energy investment that happens in many wild species. In our point of view, spermatogenesis cost is underestimated especially when it happens during harsh environmental conditions, which introduces a bias in the ecological niche framing sex-specific biological energetics. With the tenet of gestation and lactation being energy-consuming processes, females are traditionally thought to be naturally more challenged with a higher fuel need to supply than the males. But with a high spermatogenesis rate, energy allocation for reproduction is probably very costly for male mouse lemurs also (Harcourt et al., 1981; Aslam et al., 2002), as for other species involved in sperm competition.

The ecological "niche" in which animals enter an active metabolic status can be drastically different according to sex (Darwin, 1874; Shine, 1989) and expose males and females to unequal challenges. As males use their fat storage in winter to produce high quantities of gametes in anticipation of the mating season, they are in an unbalanced situation compared to females at the beginning of summer. They thus strongly depend on good environmental conditions and food availability after the main energetic investment, when females can use their energy reserve if an unpredicted food shortage happens during gestation or lactation. Pregnant female bats were recorded to maintain normothermic body temperatures during spring migration and suffer consistently less water loss than males, which entered torpor more frequently (Cryan, 2003). This would tend to dig the gap between males and females on their ability to survive summer, and introduce a "loss of opportunity" to survive in male mouse lemurs. Indeed, field data gather evidence that goes in the direction of a rougher summer for males, with a survival 16% inferior than the one of females' at the beginning of the season (Kraus et al., 2008). It is explained in this paper by the "risky male" theory, where males face dangerous situations during territorial and dispersal behaviors at the early stages of summer during mating. Emphasizing this, male-biased predation seems to be common in nature and is largely documented (see review Christe et al., 2006). This behavioral-based hypothesis does not take into account the energy balance ensuing from physiological mechanisms linked to reproductive activity in males and the rise of testosterone levels, which could be very costly in terms of metabolic activity, immunity and thus fitness (Muehlenbein and Bribiescas, 2005). Moreover, habitat and food resources use is highly influenced by dominance relationships specific of mouse lemurs, which are in favor of females (Radespiel et al., 1998; Hohenbrink et al., 2015). Which of these processes, whether behavioral, physiological or ecological are of primary importance in the field, remains an open question.

CONCLUSIONS

We found evidence of sex-specific regulation of metabolic activity in mouse lemurs submitted to CR at the end of summer, which could compel with their own programmed physiological and behavioral agenda. Contrary to females, males tended to lower their metabolic activity, but they began the experiment with a lower body mass than females and still lost similar amount of weight. Moreover, males and females seem to differ in how they regulate their oxidative status, hormones, and energy expenditure, which appear to differ under both baseline and challenging conditions. In other words, these sex differences might point to various selective pressures across the life stages acting on the physiological machinery of males and females that need to optimize their functions to expression of life-history traits. Although our results came from animal maintained under captive conditions, they suggest that the survival probability until the next season of males is much more dependent than females on the environmental conditions experienced during summer. As the climatic evolution in Madagascar is growing unpredictable with climate change (Canale and Henry, 2010), it is hypothesized that selective pressures would favor individuals with a higher body mass or with greater capacities to store fat (Dewar and Richard, 2007). Whether this could impact sex-ratio of mouse lemur populations may depend on the rapid adaptation of males to food shortage. This type of sex-specific ecological challenge is still poorly discussed and would merit more attention, especially since two-sex models of population dynamics proved that a biased-sex ratio could lead to a rapid collapse in vertebrates (Le Galliard et al., 2005; Grayson et al., 2014).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Cuvier Ethics Committee for the Care and Use of Experimental Animals of the Muséum National d'Histoire Naturelle.

AUTHOR CONTRIBUTIONS

AN, FA, and JT designed the work. AN, CR, and LP did the animal work. AN realized all the analytical experiments and statistical analyses. AN and LP did the indirect calorimetry analysis. AN, DC, and JT realized the anti-oxidant activity experiments. AN and LP did the cell culture. AN and J-FR realized the Mt/Nu DNA measurements. AN, FA, LP, and JT analyzed and interpreted the different sets of data. AN, FA, and JT wrote the manuscript. AN, DC, FA, JF-R, and JT reviewed the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00506/full#supplementary-material

Figure S1 | Comparison of **(A)** Females and **(B)** Males Principal Component Analysis. Parameters indicative of energy balance (Body mass "BM"), metabolic activity (Cortisol "Cortisol," Thyroxin "T4," Mean EE over day "EE (day),'' mitochondrial respiration (Oxydative coupling rate "OxCR," Mitochondrial Reserve Capacity "MtRC," Glycolytic Potential "GlcP"), Oxidative status (8-OHdG "8-OHdG," Thiols "Thiols," Glutathione Peroxydase "GPx"), sexual hormones (Estradiol "Estradiol," Testosterone "Testosterone") were included in the analyses. Individual plots are shown in females **(A)** and males **(B)** and grouped into caloric treatment (CTL in red, 60%CR in green). The contribution of each parameter is also represented by black arrows displaying the "Variable factor map". **(C)** Table of correlation coefficients and corresponding p-values shows only the variables significantly contributing to the two first principal components (PC1 and PC2) of the analysis (Quantitative) and the discrimination of qualitative variables: CTL and 60%CR. $^{*} < 0.05$, $^{**} < 0.01$, $^{***} < 0.001$.

Figure S2 | Correlation networks of females and males parameters. Data before and after caloric restriction were all included in the analysis for each parameter and each sex. Each parameter is written in black (Body Mass, Glycaemia, T4, Cortisol, 8OHdG, Thiols, Gpx, Testosterone, Estradiol, mean VO2, EE and RER during day or night). The closer one variable is to another, the higher the relationship. The opposite is true for widely spaced variables. Blue lines represent positive correlations (only correlations from $r = 0.5$ to 1 appear, with $p < 0.1$), while red lines negative ones. The line shade and thickness modelize the strength of the relationship.

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Countergradient Variation in Reptiles: Thermal Sensitivity of Developmental and Metabolic Rates Across Locally Adapted Populations

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Environmental temperature is a key driver of variation in developmental physiological rates in reptiles. Cooler temperatures extend development time and can increase the amount of energy required to achieve hatching success, which can pose fitness consequences later in life. Yet, for locally-adapted populations, genetic variation can oppose environmental variation across ecological gradients, known as countergradient variation (CnGV). Biologists often seek to understand the presence of phenotypic variation, yet the absence of such variation across environmental gradients can also reveal insights into the mechanisms underlying local adaptation. While evidence for genetic variation opposing environmental variation in physiological rates has been summarized in other taxa, the generality of CnGV variation in reptiles is yet unknown. Here I present a summary of studies measuring development time and metabolic rates in locally-adapted populations across thermal clines for 15 species of reptiles across 8 families. CnGV in development time is found to be common, while no clear pattern emerges for the thermal sensitivity of metabolic rates across locally-adapted populations. CnGV in development time may be an adaptive response in order to decrease the costly development in cool climates, however, empirical work is needed to disentangle plastic from genetic responses, and to uncover potentially general mechanisms of local thermal adaptation in reptiles.

Keywords: temperature, climate, adaptation, cogradient, incubation, embryo, maternal investment

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INTRODUCTION

Thermal regimes often vary considerably across spatio-temporal gradients, yet similar developmental phenotypes can be maintained when genetic variation opposes environmentally-induced variation (Levins, 1969; Conover and Schultz, 1995). Biologists have long sought to understand sources of phenotypic variation along thermal gradients, such as genotype-environment co-gradient variation (CoGV) that occur when genotypes non-randomly and positively affect phenotypes across environments (**Box 1**). Yet geographic variation in genotypes can also oppose environmental effects, thereby reducing, or masking observable phenotypic variation across a species thermal range (Taylor et al., 2015). This form of countergradient variation (CnGV) in thermally-sensitive traits such as physiological rates is important because it likely reflects an adaptive response, whereby selection acts to reduce phenotypic variance across

BOX 1 | Genotype-environment covariances: co- and counter-gradient variation. Genotype-environment covariances [Cov(G,E)] can be either positive or negative, depending on whether they reinforce or oppose each other. There are three potential ways in which genotype-environment covariances can play out across populations. First, genotypes (G) and the environment (E) shift trait expression in the same direction, known as cogradient variation where the Cov(G,E) term is positive (e.g., **Figures 1B,E**). Second, trait shifts due to genotypes do not align with trait shifts due to the environment [Cov(G,E) is negative], referred to as countergradient adaptation (e.g., **Figures 1C,F**). Alternatively, phenotypes that arise from genotypes distributed randomly in a population that change only in response to the environment, are the result of phenotypic plasticity (e.g., **Figures 1A,D**).

environmental gradients in response to local selection regimes. Thus, investigating patterns of phenotypic uniformity in nature, rather than just phenotypic variability, can help us to understand potentially general mechanisms underlying local adaptation.

There are many instances of both co- and counter- gradient variation among populations spanning a range of taxa, where natural selection drives variation across spatial and temporal thermal gradients, from microclimate and seasonal shifts, to species-level altitudinal and latitudinal ranges, or climatic shifts (Conover et al., 2009). Physiological rates often show countergradient variation: in a review by Conover et al. (2009), 87% of the studies showing evidence for CnGV were for measures of growth and developmental rates, while evidence for CoGV in physiological rates was found to be comparatively rare (Kelly, 2019). It is unclear why CnGV in the thermal sensitivity of physiological traits, relative to other traits, is so prevalent, however, it may be due to relatively weaker genetic constraints in physiological traits [compared with for example, the often strong genetic constraints of morphological traits which generally show CoGV (Li et al., 2011)]. Temperature poses a strong influence on physiological rates underlying energy acquisition and utilization in ectotherms that often misalign with the direction of selection. For example, an acute decrease in environmental temperature increases development time, yet cold climates often select for faster development so that embryos can complete development and commence feeding and growth before the onset of winter (Edge et al., 2017). CnGV can enable populations to compensate for the direct effects of temperature on physiological rates, to ensure persistence of populations under extreme climatic regimes (Angilletta, 2009; Conover et al., 2009).

In egg laying species, temperature experienced during embryonic development can impart significant fitness consequences, either through hatching success (survival) or effects imparted later in life, for example reductions in size at hatching, growth rates and reproductive success (Warner et al., 2010; Andrews and Schwarzkopf, 2012; DuRant et al., 2013; Ospina et al., 2018). Low temperatures can affect key physiological rates during development, including increasing time from fertilization to hatching (development time) and decreasing rates of energy expenditure (metabolic rate). Across a species thermal range, it is reasonable to assume both development time and metabolic rate are under stabilizing selection since adequate time and energy is needed to successfully complete cell division and differentiation, however, shifts from the optima in either trait could expose embryos to higher mortality risk via predation, desiccation, or depletion of energy reserves (Martin et al., 2007; Burton et al., 2011; Nord and Nilsson, 2011).

Combined, the thermal sensitivities of developmental and metabolic rates determine how energy use during development (fertilization until nutritional independence) scales with temperature (Pettersen et al., 2019). Increasing either development time (D), or metabolic rate (MR) will increase the costs of development, and therefore reduce the amount of residual energy at hatching. The recently proposed Development Cost Theory (DCT) posits that the relative temperature sensitivities of D and MR determine the amount of energy expended at any given temperature (Marshall et al., 2020). At cooler developmental temperatures, D is often increased more than MR decreases, hence cold environments generally increase total energy use, thereby reducing energy available for fitness-enhancing processes such as growth, maintenance and foraging (Booth and Thompson, 1991; Angilletta et al., 2000; DuRant et al., 2011; Pettersen et al., 2019), however, there are exceptions (e.g., Oufiero and Angilletta, 2010). Based on DCT, the temperature dependence of developmental rate, has the greatest influence on the relative costs of development, and is therefore expected to evolve more rapidly than the thermal sensitivity of metabolic rate (Marshall et al., 2020). DCT can thus provide a useful framework for detecting local adaptation by providing a mechanistic link between population-level reaction norms and fitness across thermal gradients.

While development time and the costs of development are generally increased at low environmental temperatures, countergradient variation can compensate for these effects. Countergradient variation can reduce the costs of development associated with cool temperatures via variation in developmental and metabolic rates that oppose the acute effects of environment on phenotype – for example, higher physiological rates can be maintained despite decreases in environment temperature. In order to identify whether the genetic component for the change in mean D or MR is statistically correlated with thermal gradient, three criteria must be met: (1) measures of the pattern of change in D and MR across a spatially or temporally varying environmental gradient; (2) the norm of reaction for D and MR in response to temperature; (3) a measure of the magnitude of thermal variation across the gradient. It is often difficult to unequivocally demonstrate that inter-populational divergence in thermal sensitivity of a trait is a result of adaptive genetic divergence, and not due to a plastic response. Obtaining evidence for CoGV and CnGV requires collecting data from common garden or reciprocal transplant studies, conducted across a range of temperatures in order to distinguish between V_{GxE} and Cov(G,E) (Yamahira and Conover, 2002; Yamahira et al., 2007). Norms of reaction that are parallel, and those that lie above or below one another in trait value provide evidence for CnGV and CoGV, respectively. Whereas, both V_{GxE} and Cov(G,E) are acting simultaneously when norms of reaction are not parallel and do not cross (see Box 2 in Conover and Schultz, 1995). While the prevalence of CoGV and CnGV in physiological traits has been reviewed in fish (Conover et al., 2006), amphibians (Morrison and Hero, 2003), marine invertebrates (Sanford and Kelly, 2011), and insects (Sinclair et al., 2012), examples in reptiles are less well documented. This is surprising, given that reptiles represent one of the largest study groups in vertebrate thermal physiology.

It is important to develop a clear understanding of patterns of countergradient variation in nature, before designing experiments to evaluate causal mechanisms (Conover et al., 2009). This paper therefore compiles data from common garden (CG) and reciprocal transplant (RT) studies testing for temperature-by-population interactions on variations in development time (*D*) and metabolic rate (*MR*) across cold- and warm-adapted populations of reptiles (Li et al., 2018a). Effect sizes for each study, weighted by sample size can then be calculated in order to test whether selection has modified reaction norms of *D* and *MR* across climatic regimes (**Supplementary Table S1**). It is anticipated that despite a decrease in environmental temperatures, cold-adapted populations maintain similar *D* and (or) *MR* across a species' thermal gradient, relative to warm-adapted populations. Reptiles provide a useful model system to study local adaptation because developmental trajectories in reptiles are highly sensitive to environmental temperatures (Angilletta, 2009), and many reptile species have limited dispersal ability between populations (Uller and While, 2015). This review aims to elucidate broad-scale mechanisms underlying local adaptation in reptiles by evaluating the generality of phenotypic plasticity [$\mathrm{Cov}(G,E) = 0$; **Figures 1A,D**], cogradient variation [$\mathrm{Cov}(G,E) > 1$; **Figures 1B,E**], and countergradient variation [$\mathrm{Cov}(G,E) < 1$; **Figures 1C,F**] in developmental physiological rates across populations experiencing different thermal regimes. If populations maintain similarity in *D* and (or) *MR* under thermal change then evolution is likely the result of CnGV, whereas rapid trait divergence in *D* or *MR* in the direction of thermal change is due to the evolution of CoGV. Due to the paucity of data on thermal sensitivity of *D* and *MR*, it is not yet feasible to present a formal, comprehensive meta-analysis on the topic here. Rather, this review serves as a summary of existing data on thermal reaction norms across locally adapted populations, and points toward future avenues of research that require further work in order to continue developing our understanding of adaptation along thermal gradients.

Countergradient Variation of Thermal Sensitivity in Reptiles Is Prevalent in Developmental but Not Metabolic Rates

Most published studies show evidence for CnGV between development time and environmental temperature [negative values of Hedges' *g* (*D*); **Figure 2**], supporting the generality of countergradient variation in reptile development. For 17 out of 22 studies, intrinsic (genetic) factors were shown to counter thermal influences on developmental rate. Rather than an outcome of genetic drift, these findings suggest an adaptive countergradient response – selection opposes reaction norms of development time (*D*) across climatic regimes. Development under cool conditions necessitates a countergradient adaptive response for faster development and earlier hatching time, enabling embryos to hatch before winter while resources are still available (Du et al., 2012). On the contrary, there is little support to suggest that CnGV is common for metabolic rate (*MR*) – overall, reptile embryos from locally-adapted cooler climates did not maintain higher metabolic rates compared with populations from warmer climates (**Figure 2**). This could be due to several reasons, not least that metabolic rate is a highly variable trait, even after correcting for mass and temperature (Burton et al., 2011). There is also evidence for population-level differences in thermal reaction norms in heart rate across development stage (Angilletta et al., 2013). Since metabolic rate is not a single trait (Pettersen et al., 2018), multiple measures of *MR* throughout development are needed in order to elucidate patterns in rates of energy expenditure across locally-adapted populations.

Despite an apparent lack of evolved response in MR to buffer against reduced energy turnover under cold temperatures, a countergradient response in *D* can itself reduce energy expenditure during development under cold conditions. Across a species' natural temperature range, the thermal sensitivity of *D* is often greater than the thermal sensitivity of *MR* and is therefore a more important determinant of how the costs of development scale with temperature (Pettersen et al., 2019; Marshall et al., 2020). It may be that embryos counteract increased energy costs associated with development under cold temperatures, by reducing *D* without a concomitant increase in *MR*, and is supported by evidence for CnGV in yolk assimilation in the eastern fence lizard (Storm and Angilletta, 2007). The ability to evolve increases in one physiological rate independently of another has been shown previously for metabolic and growth rates (Williams et al., 2016). It may be that for developmental physiological rates, selection acts to reduce the costs of development, via CnGV in the thermal sensitivity of *D*, resulting in a closer alignment of embryo thermal optima to that of local thermal regimes.

Proximal Drivers of Countergradient Adaptation in Developmental Rates

Various mechanistic explanations have been proposed to explain the prevalence of countergradient variation in developmental rates, and its compensating effects on the costs of development at cooler temperatures, including the role of maternal effects. It is a common view that faster development in cold-, vs. warm-adapted populations reared under common garden conditions are a consequence of later stage of embryogenesis at laying, earlier stage of development at hatching, or larger egg size. For example, extended embryo retention and greater maternal provisioning in order to reduce *D* is often associated with cool climates in squamate reptiles, both among and within species (Shine, 1995; While et al., 2015). Yet, even after accounting for population-level differences in maternal investment, studies find faster developmental rates in cold-adapted populations (Storm and Angilletta, 2007; Du et al., 2010). Increased heart mass in cold-adapted populations may allow for faster cell division and differentiation associated with development (Du et al., 2010), however, how these effects are isolated from

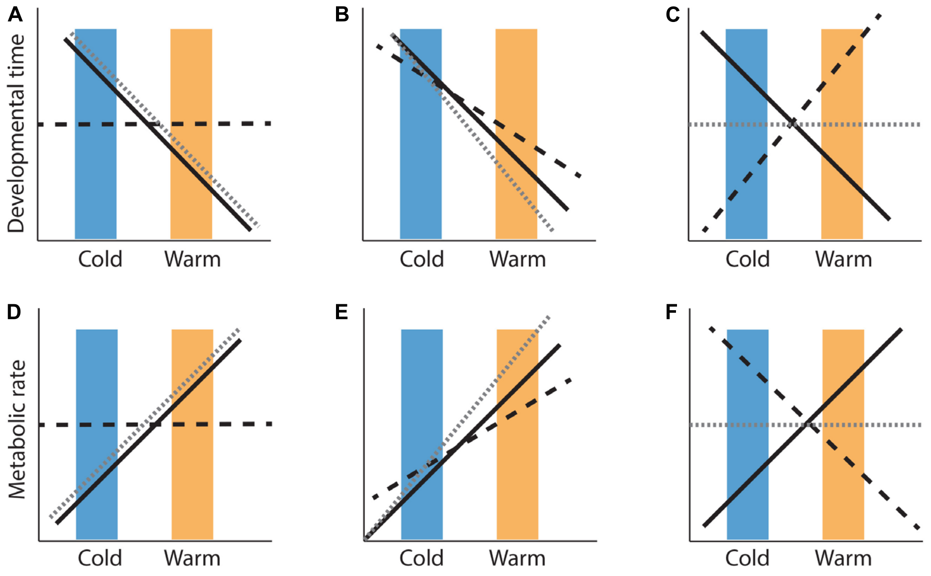

FIGURE 1 | Hypothetical phenotypic variation (gray dashed lines) across locally-adapted cold (i.e., non-native conditions; blue boxes) and warm (i.e., native conditions; orange boxes) populations as a result of environmental temperature (black solid lines) and genetic (black dashed lines) effects. Shifts in phenotypic values of development time **(A–C)** and metabolic rate **(D–F)** in response to environmental temperature, can be entirely determined by environment (phenotypic plasticity; **A,D**) where the covariance between genetic (*G*) and environmental (*E*) effects = 0. Alternatively, genotypic differences can be in the same direction as environmental influences [positive Cov(*G,E*)], amplifying environmental effects on phenotypic (co-gradient variation; **B,E**), or they can oppose environmental temperature effects [negative Cov(*G,E*)], resulting in little or no phenotypic change across cold and warm environments **(C,F)**. Note, reaction norms may differ among genotypes, posing environmentally determined effects on phenotype value [V_{GXE}; discussed in Box 2 in Conover and Schultz (1995), not shown here].

effects on the thermal sensitivity of heart rates is difficult to explain. Variation in yolk hormone content and composition (Ruuskanen et al., 2016) or enzymatic activity (Rungruangsak-Torrissen et al., 1998) may also play a role in facilitating faster developmental rates in cold-, relative to warm-adapted populations. Indeed, there are likely to be multiple mechanisms responsible for countergradient variation in *D*, rather than any single factor.

Consequences of Countergradient Adaptation: When and Why Is Thermal Countergradient Adaptation Absent?

Despite the prevalence of CnGV in development time, there are studies that do not show this trend, for example evidence for CnGV was absent across native-non-native ranges for species adapting to hot temperatures. When comparing forested (cool) vs. urban (hot) populations of *Anolis cristatellus* and *A. sagrei* under common garden conditions, co-gradient variation was observed where hot-adapted populations showed shorter development times relative to warm-adapted populations (Tiatragul et al., 2017; Hall and Warner, 2018). These findings are congruent with CDT – beyond a species usual temperature range development is more costly because metabolic rate increases more than development time decreases (Marshall et al., 2020). Decreasing *D* at hot temperatures results in reduced costs of development, and therefore likely fitness advantages. Further measures of the relative temperature dependencies of *D* and *MR* in other species are needed to elucidate the temperature-dependent costs of development as a potentially general mechanism for local thermal adaptation to extreme high temperatures.

Trade-offs with other fitness-related traits can also help to explain an absence of CnGV in *D* – reducing development time may come at the cost of embryos hatching at smaller sizes and reduced juvenile growth rate (Angilletta et al., 2003; Buckley et al., 2010). However, in reptiles, evidence for trade-offs amongst life-history traits are mixed (Niewiarowski and Angilletta, 2008; Fetters and McGlothlin, 2017). Alternatively, it may be that selection on other traits can compensate for a lack of CnGV in developmental physiological rates. In squamates, behavioral thermoregulation, such as shifts in female body temperature while gravid, may be a more labile, and therefore more important mechanism for adaptation to cold and variable climatic regimes than perhaps more conserved, physiological responses (Navas, 2002).

Other climatic factors that vary across thermal gradients, such as temperature variation, seasonal time constraints, and food availability may confound effects of temperature on developmental rates. Studies using fluctuating, rather constant temperature manipulations showed mixed results, such as the absence population-level patterns (Angilletta et al., 2013) or evidence for CoGV (Li et al., 2018b). Both CoGV and CnGV were found under temperatures that were fluctuating, but representative of natural nest temperatures (Oufiero and Angilletta, 2006; Li et al., 2018b; **Figure 2** and **Supplementary Table S1**). In order to capture realistic, population-level

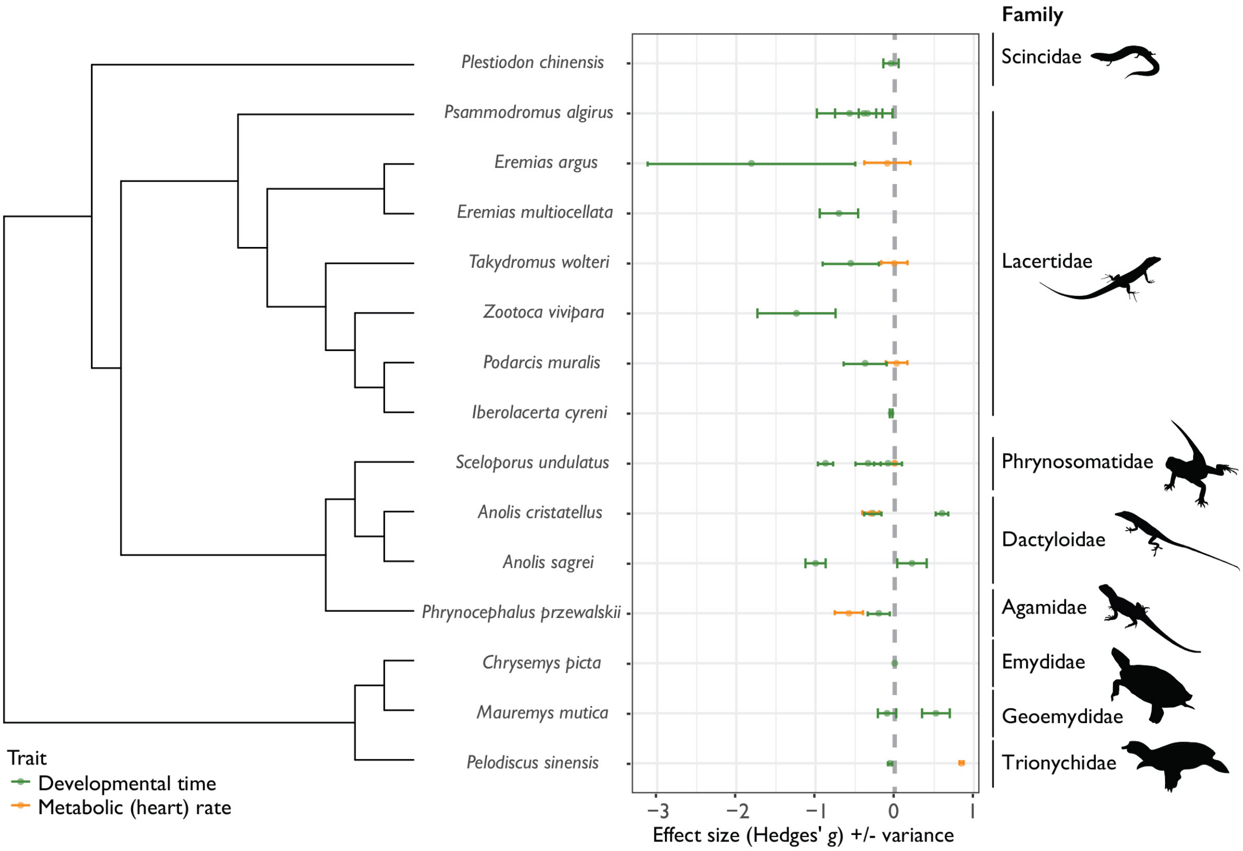

FIGURE 2 | Effect sizes (Hedges' *g*) for differences in the thermal sensitivity of development time (time from oviposition until hatching) and metabolic (heart) rate across cold and warm-adapted populations for 15 species of reptiles across 8 families (± variance). For development time (*D*; green data points and variance bars), positive Hedges' *g* values indicate positive Cov(*G,E*), or cogradient variation, where cold-adapted populations have longer *D*, relative to warm-adapted populations. Negative values of *D* indicate negative Cov(*G,E*), or countergradient variation, where genotypic differences oppose environmental temperature effects – in these instances, cold-adapted populations develop faster than warm-adapted populations. For metabolic rates (*MR*; orange data points and variance bars), negative Hedges' *g* values indicate positive Cov(*G,E*), or cogradient variation, where cold-adapted populations have lower *MR*, relative to warm-adapted populations, while positive values of *MR* indicate negative Cov(*G,E*), or countergradient variation – here cold-adapted populations maintain higher *MR*, relative to warm-adapted populations.

responses, it is important that temperature manipulations reflect natural thermal conditions that can account for non-linearity in the thermal dependence of physiological rates (Denny, 2017). Further, thermal fluctuation studies offer complex, albeit vital insights into whether CnGV can be maintained under rapid environmental change, and more studies are needed to reveal any consistent patterns across magnitudes of spatial and temporal thermal gradients (Du et al., 2010; Li et al., 2018a).

Finally, it may be that interactions between genotype and environment are inflating observations of CnGV across thermal regimes, and that CnGV in development time is less common than currently assumed. There may be genotype-dependent effects of environment on development time, where for example, a single genotype is superior in all environments, even though the slopes of reaction norms differ (see Box 2 in Conover and Schultz, 1995). All studies included in this review only observed a single generation – it is unlikely that all sources of V_E are controlled for over this timescale (plastic responses may still play a role), which is a limitation of studying species with relatively long generation times, such as reptiles and other vertebrates (Laugen et al., 2003).

Future Directions for Understanding Local Adaptation via Evolution of Developmental Rates in Reptiles

Adaptation of developmental physiological rates is an important, yet underutilized avenue of research for understanding population persistence under changing and novel environments. Countergradient variation for traits expressed later in the life history have been well documented in reptiles, including growth (Sears and Angilletta, 2003; Uller and Olsson, 2003; Li et al., 2011; Snover et al., 2015; Ortega et al., 2017), body size (Oufiero et al., 2011; Iraeta et al., 2013), scale size (Oufiero et al., 2011), preferred body temperature (Hodgson and Schwanz, 2019), nest date (Knapp et al., 2006; Edge et al., 2017), reproductive output (Knapp et al., 2006; Li et al., 2011; Fetters and McGlothlin, 2017), critical thermal limits and water loss (Kolbe et al., 2014) and locomotor performance (Niewiarowski, 2001; McElroy, 2014). It is possible that CnGV for traits observed later in life are also a consequence of developmental environment, such as food availability, temperature and stress (DuRant et al., 2013; Noble et al., 2018).

Developmental and metabolic rates under selection may in turn affect selection on genetically correlated traits later in life (Artacho et al., 2015; Pettersen et al., 2016, 2018; Ricklefs et al., 2017). Resolving the interplay between plastic and genetic responses to local selective forces throughout the life history and environmental gradients is the next fundamental challenge (Buckley et al., 2010). Isolating the role of maternal effects from environmental effects can be challenging without multi-generational studies, nevertheless, investigating the effect of maternal environment on offspring phenotype can provide insight into mechanisms underlying rapid adaptation to novel environments. Variation in maternal investment along environmental gradients is common in reptiles, even in egg-laying species that buffer their offspring from external temperatures via behavioral (Mathies and Andrews, 1996; Du et al., 2010), physiological (Harlow and Grigg, 1984) or endocrinological (Uller et al., 2007) mechanisms which may complement or even drive countergradient variation to facilitate acclimation and adaptation to local thermal regimes.

CONCLUSION

Across latitudinal and altitudinal clines, cold-adapted populations have genetic capacity for faster development, relative to warm-adapted populations. While these differences in thermal sensitivity to local temperatures did not extend to warm- vs. hot-adapted populations such as forested vs. city populations, there is overall support for common CnGV in development time in reptiles, which mirrors findings observed in other taxa (Conover et al., 2009). Given the highly sensitive nature of developmental trajectories to acute changes in temperature, maintenance of stable physiological rates in species covering wide distributions offers a fascinating avenue for understanding local adaptation (Du et al., 2010). In particular, evolutionary change in the thermal sensitivity of developmental and metabolic rates is likely to be a crucial component of adaptive responses to environmental change (Kelly, 2019). Identifying the nature of genotype-environment covariances across ecological gradients is key to understanding variation in physiological rates and for predicting population persistence under environmental change.

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SUPPLEMENTARY MATERIAL

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ORIGINAL RESEARCH
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Increased Diurnal Activity Is Indicative of Energy Deficit in a Nocturnal Mammal, the Aardvark

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Shifting activity to cooler times of day buffers animals from increased heat and aridity under climate change. Conversely, when resources are limited, some nocturnal species become more diurnal, reducing energetic costs of keeping warm at night. Aardvarks (*Orycteropus afer*) are nocturnal, obligate ant- and termite-eating mammals which may be threatened directly by increasing heat and aridity, or indirectly by the effects of climate change on their prey. We hypothesised that the minimum 24-h body temperature of aardvarks would decline during energy scarcity, and that aardvarks would extend their active phases to compensate for reduced resource availability, possibly resulting in increased diurnal activity when aardvarks were energetically compromised. To measure their thermoregulatory patterns and foraging activity, we implanted abdominal temperature and activity data loggers into 12 adult aardvarks and observed them for varying durations over 3 years in the Kalahari. Under non-drought conditions, aardvarks tightly controlled their 24-h body temperature rhythm (mean amplitude of the 24-h body temperature rhythm was 1.8 ± 0.3°C during summer and 2.1 ± 0.1°C during winter) and usually were nocturnal. During a summer drought, aardvarks relaxed the precision of body temperature regulation (mean 24-h amplitude 2.3 ± 0.4°C) and those that subsequently died shifted their activity to progressively earlier times of day in the weeks before their deaths. Throughout the subsequent winter, the aardvarks' minimum 24-h body temperatures declined, causing exaggerated heterothermy (4.7 ± 1.3°C; absolute range 24.7 to 38.8°C), with one individual's body temperature varying by 11.7°C within 8 h. When body temperatures were low, aardvarks often emerged from burrows during daytime, and occasionally returned before sunset, resulting in completely diurnal activity. Aardvarks also shortened their active periods by 25% during food scarcity, likely to avoid energetic costs incurred by foraging. Despite their physiological and behavioural flexibility, aardvarks were unable to compensate for reduced food availability. Seven study aardvarks and several others died, presumably from starvation. Our results do not bode well for aardvarks facing climate change, and for the many animal species dependent on aardvark burrows for refuge.

Keywords: body temperature, heterothermy, biologging, behavioural flexibility, climate change

INTRODUCTION

Large mammals that reproduce slowly and are unable to move to more suitable surroundings require flexibility in behaviour and physiology to survive in rapidly changing environments (Fuller et al., 2016). Understanding flexibility in activity patterns of mammals is crucial for predicting their resilience to climate change (McCain and King, 2014). Many mammals buffer the effects of increasing heat and aridity, occurring with climate change, by seeking cooler microclimates and reducing diurnal activity (Hetem et al., 2012a; McFarland et al., 2014). Those adjustments reduce the demand for water for evaporative cooling. If cooler microclimates are not available or if reduced diurnal activity compromises energy intake, diurnal species may increase their nocturnal activity (Hetem et al., 2012a; Levy et al., 2019).

Nocturnal species already are active at the cooler time of day, so one might predict less change in their activity patterns in response to increasing heat and aridity. By contrast with diurnally active mammals that might need to increase evaporative water loss to keep cool, mammals active during the night might need to increase their metabolic rates to keep warm, which may impose an extra demand for energy during cold nights. Indeed, increasing air temperatures at night, as a result of climate change, might benefit nocturnal species by reducing the energetic costs of maintaining homeothermy (Levy et al., 2019), a benefit that might help offset the reduced nutritional quality or availability of food resulting from climate change (Durant et al., 2007; Post et al., 2008; Birnie-Gauvin et al., 2017). In environments with clear night skies, substantial heat loss occurs through passive radiant cooling to the night sky, which acts as a heat sink with an effective temperature of between −10 and −30°C (Mitchell et al., 2018). Because the radiant heat sink of the night sky is unlikely to change much with climate change, warmer air temperatures at night are unlikely to offset the energetic cost of reduced resource availability. Instead, as resources become limited, the ability to keep warm at night using metabolic heat might be compromised, such that some nocturnal species may increase their diurnal activity to reduce the energy cost that would have been incurred through being active at night (Van der Vinne et al., 2019). The trade-off then is that they have to acquire sufficient resources while exposed to daytime heat loads, with air temperatures increasing under climate change.

Alterations in activity patterns across the day, as well as changes in energy or water balance, have implications for body temperature regulation. Periods of energy deficit resulting from reduced food availability or increased energy expenditure are associated with low minimum 24-h body core temperatures (Fuller et al., 2014; Hetem et al., 2016) for many large mammal species (Maloney et al., 2011; Signer et al., 2011; Brinkmann et al., 2012; Hetem et al., 2012b; Lubbe et al., 2014; Whiteman et al., 2015). On the other hand, large mammals exposed to water deficit in hot and dry environments exhibit high maximum 24-h body temperatures (Mitchell et al., 2002; Hetem et al., 2010; Fuller et al., 2014), thereby reducing water loss through evaporative cooling. Such increases in the maximum 24-h and decreases in the minimum 24-h body temperatures result in increases in the amplitude of the 24-h body temperature rhythm. Because an increased amplitude of the 24-h body temperature rhythm, termed heterothermy, is associated with an energy deficit or water deficit or both, it reflects a disruption of the physiological well-being of large mammals in their natural environment (Fuller et al., 2014). Indeed, homeothermy appears to occur only in healthy, undisturbed large mammals with sufficient access to food and water (Hetem et al., 2016).

One species that exhibits increased heterothermy in the face of heat and aridity is the aardvark, *Orycteropus afer* (Rey et al., 2017). Aardvarks are solitary, burrowing mammals that occur in non-desert habitats of sub-Saharan Africa. They extract ants and termites, on which they depend for energy and water, by digging them out of their underground hives (Taylor, 1998; Weyer, 2018). Aardvarks generally emerge from their burrows at night to forage, but demonstrate flexibility in behaviour and activity patterns in response to environmental fluctuations. Aardvarks in the semi-arid Karoo region of South Africa displayed an increased amplitude of their 24-h body temperature rhythm during winter when it was cold, as a result of low minimum 24-h body temperatures (Taylor and Skinner, 2004), whereas aardvarks in the more-arid Kalahari region of South Africa displayed an increased amplitude of the 24-h body temperature rhythm during a summer drought, as a result of low minimum 24-h body temperatures (Rey et al., 2017). In both studies, the low body temperatures were accompanied by an increase in diurnal activity. In the Kalahari, many aardvarks (including five out of six study aardvarks) died during the drought, presumably from starvation, and survivors were in poor condition (Rey et al., 2017). It remains unclear whether the shift to diurnal activity resulted from aardvarks being intolerant of the cold night-time temperatures in winter (Taylor and Skinner, 2004), or from an attempt to reduce the energetic costs of homeothermy when resources were limited (Rey et al., 2017). Here, we expand our previous study to investigate the key drivers of changes in body temperature and activity in aardvarks.

We assessed the consequences of fluctuating resource availability and environmental variables on body temperature rhythms and activity patterns of aardvarks in the Kalahari. We hypothesised that minimum 24-h body temperature of aardvarks would decline during periods of low food resource availability, resulting in an increased amplitude of the 24-h body temperature rhythm. Secondly, we hypothesised that to compensate for reduced food availability or increased energetic cost to maintain homeothermy during cooler times of the year (in winter), aardvarks might become increasingly diurnal when they were more compromised energetically.

MATERIALS AND METHODS

Study Site

The study was carried out between July 2012 and September 2015 at Tswalu, a private nature reserve in the Northern Cape Province of South Africa (S 27°14′, E 22°22′). The dominant vegetation type comprised Kalahari thornveld (Acocks, 1988) and shrubby Kalahari dune bushveld on the plains (Low and Rebelo, 1998). The aardvark study site was dominated by Gordonia duneveld vegetation (Tokura, 2016). Large carnivores present were leopards (*Panthera pardus*) and cheetahs (*Acinonyx jubatus*). African wild dogs (*Lycaon pictus*) were introduced in 2014. Tswalu is situated within an area that is predicted to become hotter and drier under future climate change (Engelbrecht et al., 2015), and already experiences low and highly variable rainfall, including sporadic droughts (Thomas et al., 2007). The majority of rain falls between December and April (Davis et al., 2010; Tokura, 2016). The hot season in which air temperatures peak at ∼35°C lasts from November to February, which overlaps with the period during which rainfall typically occurs. The cold season in which air temperatures frequently drop below zero lasts from June to September and typically coincides with the driest period.

Climatic Variables

We recorded the air temperature at the study site at 30-min intervals using a weather station (Watchdog 2700, Spectrum Technologies Inc., United States; data provided by A. Young, Exeter University). From these data, we calculated for each 24-h period the minimum, maximum, and mean air temperatures. We assessed local long-term rainfall over the 33 years, including 30 years preceding our study (1982–2015; each year starting in July and ending the following June) using FLDAS_NOAH010 data provided via the online data system Giovanni, the Goddard Earth Sciences Data and Information Services Center (GES DISC) Interactive Online Visualization and Analysis Infrastructure, developed and maintained by the NASA GES DISC[1] (Acker and Leptoukh, 2007). During our study, we recorded rainfall on the study site using an event logger (HOBO Pendant Event data logger, Onset Computer Corporation, United States) and tipping bucket (Davis Instruments Corporation, United States). To assess rainfall during the growing season before our study started, we obtained rainfall data from the rainfall database of Tswalu. The two datasets showed high levels of agreement during overlapping measurement periods. We calculated total rainfall per month for the period January 2012 to December 2015. For the same period, we obtained daily times of sunrise and sunset for the nearest town (Upington, situated 200 km southwest of the study site[2]), from which we calculated the daily photoperiod.

Aardvark Capture and Surgery

Over a 3.5 year period, 12 individual aardvarks were darted and implanted with data loggers (see below) to record their body temperature and activity. At different timepoints throughout the study, our sample size ranged between one and five individuals for body temperature and one and four individuals for activity, as a result of aardvark deaths and failure of biologging devices (see **Supplementary Table S1** for details). In July 2012, we instrumented six aardvarks; five of these aardvarks died in March 2013 during the summer drought (Rey et al., 2017) and we retrieved biologgers from four of their carcasses (*A01*, *A02*, *A04*, and *A05*). In July 2013, we recaptured the surviving aardvark (*A06*), and instrumented six additional aardvarks (aardvarks *A07–A12*). One aardvark (*A10*) died during the winter of 2013. One aardvark (*A11*) died in the early summer of 2014, nearly 17 months after implantation, with no prior signs of body condition deterioration or weakness. In July 2014, we removed and reimplanted biologgers into two aardvarks (*A07* and *A08*), and one aardvark (*A12*) was added to the study in July 2014 for the third study year (July 2014 to September 2015; see **Supplementary Table S1** for details). Another died in winter 2015 (*A09*), after its body condition deteriorated. Aardvark body mass during capture averaged 35 kg (range 26.5–43.0 kg). Determination of sex was not done for the aardvarks captured before 2013. All aardvarks captured after 2013 (*A06–A12*) were females, as classified based on a published description of aardvark genitalia (Pocock, 1924).

We captured and immobilised our aardvarks as previously described (Rey et al., 2014). Each aardvark was immobilised using a propelled dart administered intramuscularly. Each dart was filled with a combination of 150 mg ketamine, 4 mg medetomidine, and 10 mg midazolam using concentrated drugs (ketamine: 200 mg mL^{-1}; medetomidine: 50 mg mL^{-1}; and midazolam: 50 mg mL^{-1}; Kyron Laboratories, South Africa), and topped up with sterile water to reach a final volume of 2 mL. Anaesthesia was maintained by inhalation of isoflurane (Isofor, 0.5–6%, SafeLine Pharmaceuticals, South Africa) in 100% medical oxygen. During surgery, each aardvark received a ringer's lactate drip administered at a maintenance rate of 5 mL kg^{-1} h^{-1}. We monitored respiration (respiratory rate, peripheral haemoglobin oxygen saturation, and end-tidal carbon dioxide) and cardiovascular (pulse rate and arterial blood pressure) variables, and rectal temperature throughout anaesthesia. A ∼100 × 100 mm section of the paralumbar region of each aardvark was shaved, washed and sterilised using antiseptic solutions (chlorhexidine: 5%, chlorhexidine gluconate: 0.5% in alcohol, F10 Products, Health and Hygiene, South Africa). Local anaesthetic (lignocaine hydrochloride: 2%, 3 mL, Bayer Animal Health, South Africa) was injected subcutaneously at the surgical site. Additionally, each aardvark received a long-acting antibiotic intramuscularly

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[1] https://giovanni.gsfc.nasa.gov/giovanni/

[2] http://staging.timeanddate.com

(Duplocillin, procaine benzylpenicillin: 6,000 IU kg^{-1}, Intervet, South Africa), a non-steroidal anti-inflammatory (meloxicam: 0.5 mg kg^{-1}, Boehringer Ingelheim Pharmaceuticals, South Africa) and an opioid analgesic (buprenorphine hydrochloride: 0.01 mg kg^{-1}, Reckitt Beckinser Healthcare, United Kingdom) subcutaneously.

A 30–50 mm incision was made through the skin, muscle layers, and parietal peritoneum, through which the temperature data logger and very high frequency (VHF) transmitter (see below) were inserted into the abdominal cavity. The data logger that recorded locomotor activity of the aardvark was inserted and tethered intramuscularly (*Musculus transversus abdominis*) using non-absorbable polyamide suture (Nylon, Scimitar Surgical Sutures, Gabler Medical, United Kingdom), to ensure that the activity records were not affected by free movement of the logger inside the animal's body. Incisions were closed using absorbable polyglycolic acid suture material (Viamac, Scimitar Surgical Sutures, Gabler Medical, United Kingdom). The wound was sprayed with a topical antiseptic (Necrospray, oxytetracycline hydrochloride: 40 mg, gentian violet: 4 mg, Animal Health Division — Bayer HealthCare, South Africa) and coated with a topical ectoparasiticide (Tick Grease, chlorfenvinphos: 0.3%, SWAVET RSA, South Africa). After surgery, each study aardvark was released as close as possible to its capture site, where a drug to reverse the immobilisation was administered slowly intravenously (Antisedan, atipamezole hydrochloride: 0.5 mg kg^{-1}, Pfizer Laboratories, South Africa). For further details about surgical procedures and data logger implantation, see Weyer (2018).

We closely observed recovery of each aardvark during the weeks following surgery. Each study aardvark was recaptured annually, and a similar procedure to that used for implants was followed to replace, or remove, the loggers and tracking transmitter. The implant sites had healed and no signs of infection from the implantation surgery were visible at recapture.

Data Loggers and Tracking Transmitter

We implanted two data loggers and one tracking transmitter into each aardvark (total mass of implanted equipment ~300 g, <1% of aardvark body mass; see **Supplementary Table S2** for dimensions of all implanted devices). Transmitters and data loggers were covered with inert wax (Sasolwax 1276, Sasol Ltd., South Africa) and sterilised in formaldehyde vapour before implantation. The tracking transmitter (Africa Wildlife Tracking, South Africa) allowed relocation of aardvarks in the field with a directional antenna and receiver kit (Three-Element Folding Yagi Antenna, R1000 Receiver, Sirtrack, New Zealand). A temperature-sensitive data logger recorded abdominal body temperature in each aardvark (2012–2013: DS1922L Thermochron iButton®, Dallas Semiconductor/Maxim Integrated Products, United States; resolution 0.0625°C; 2013–2015: DST centi-T, Star-Oddi, Iceland; resolution 0.032°C). Loggers were set to record temperature at 5-min intervals. Each temperature logger was calibrated in an insulated water bath against a high accuracy thermometer (Quat 100, Heraeus, Germany) over a temperature range of 28–42°C in increments of 2°C before implantation. We confirmed the reliability of temperature readings by re-calibrating all loggers after their removal. After calibration, the biologgers measured body temperature to an accuracy of better than 0.1°C.

Locomotor activity (see **Supplementary Table S3** for a summary) was recorded using motion-sensitive loggers. In the first year (July 2012 to July 2013), activity was recorded by tri-axial piezoelectrical accelerometers (Actical, Mini-Mitter Corporation, United States), which were sensitive to forces of >0.05 *g* and were set to record whole-body movements over full 5-min periods. In the following years (2013–2015), aardvark locomotor activity was recorded over 10-s periods at 5-min intervals using custom-designed activity biologgers (MLOG_AT1, Sigma Delta Technologies, Australia) with a tri-axial accelerometer sensitive to forces of >0.04 *g*. These devices had previously been validated against visual observations of a free-living primate (McFarland et al., 2013).

Time of Emergence and Return to Burrows

We set up camera traps (MMS wireless scouting camera, LTL-6210MC HD series, Ltl Acorn, China) outside aardvark burrows to determine the exact time of emergence from and return to a burrow after foraging (if the aardvark returned to the same burrow from which it had emerged). Between July 2013 and September 2015, we located each aardvark inside its burrow several times per month using VHF tracking, and placed camera traps at burrow entrances, either behind the burrow entrance or downwind of the burrow to minimise disturbance of the aardvark inside. Because each aardvark changed burrows frequently, we could not use a fixed camera trap system. In total, we obtained recordings of 387 emergences and 109 returns of an aardvark to a burrow (see **Supplementary Table S4** for details). We analysed all camera trap footage manually to determine time of emergence and return to burrows.

To determine whether activity loggers could reliably indicate the beginning and end of aboveground activity of aardvarks, we time-matched logger data to camera trap footage of an aardvark's times of emergence from a burrow, or an aardvark's time of return to a burrow after foraging. Occasionally after emergence from a burrow, an aardvark remained at the burrow to rest before initiating foraging; in these cases, the time of emergence was identified as the time at which the aardvark started foraging. For each data point from the activity logger, we calculated the median of six values before and six values after this data point (i.e., median activity over a duration of 65 min). If the median exceeded 5% of the maximum activity recorded for each individual, we considered the aardvark to be "active"; if not, it was considered to be "inactive." We classified the time-stamp of the data point at which the 65-min median activity level changed from "inactive" to "active" to be the time of emergence, whereas the reverse change from "active" to "inactive" marked the time of the aardvark's return to a burrow. To ensure accuracy of our calculated times of emergence from and return to burrows, we validated calculated times against the actual times obtained from camera trap footage where logger data were available at the same time as camera trap footage ($N = 176$).

Data Analyses

Some data loggers failed, thereby reducing our sample size to $n = 10$ aardvarks for body temperature and $n = 9$ aardvarks for activity data. We excluded body temperature and activity data recorded on capture days and for 2 days after capture attempts from all analyses. To account for differences in the sensitivity of individual activity loggers, each 5-min reading of activity was expressed as a percentage of the maximum activity reading (% of maximum) for each logger while implanted in a study animal (McFarland et al., 2013). For each aardvark over each 24-h period, we calculated the minimum, maximum, and amplitude (difference between maximum and minimum) of body temperature rhythm. To compare 24-h patterns of aardvark body temperature and activity during the hottest and coldest months of a drought year with a non-drought year, we calculated mean ± SD of hourly body temperature and activity recordings averaged across all aardvarks over 4 months of interest, namely February 2013 (drought summer), August 2013 (winter following drought), February 2014 (non-drought summer), and August 2014 (winter following non-drought summer). We obtained air temperature at the time of emergence and at the time of return of each study aardvark per day ($n = 1$ to 5), and averaged the air temperature coinciding with time of emergence and time of return between these aardvarks for each 24-h period.

We used a series of generalised linear mixed-effect models (GLMMs) to determine the effects of environmental variables on aardvark body temperature patterns. We used each 24-h period as a sampling unit in the GLMMs, and added a first order autocorrelation structure to account for temporal autocorrelation. We included aardvark identity as a random factor. The dependent variable was minimum or maximum or amplitude of the 24-h body temperature rhythm. Independent variables included photoperiod, minimum or maximum or range of 24-h air temperature, and vegetation greenness as a proxy of resource abundance for aardvark prey. This vegetation greenness index consisted of moderate-resolution imaging spectroradiometry (MODIS)-derived time-series enhanced vegetation index (EVI) data for the Gordonia duneveld vegetation (Tokura, 2016). EVI was measured every 16 days and averaged per month. We previously have shown that EVI as an index for aardvark prey's food availability correlated with aardvark body condition (Weyer, 2018). We tested for multicollinearity of independent variables within each model using variance inflation factors (VIF) and ensured that all VIF <2, based on a conservative cutoff (Zuur et al., 2010).

To assess changes in activity patterns, we calculated the duration of activity between time of emergence from and return to the burrows each day. On occasion, aardvarks did not emerge from their burrows and remained inactive (see **Supplementary Table S3**); foraging duration was assigned a zero value for these days. For each aardvark, we also calculated the proportion of each active phase that occurred between sunrise and sunset, to quantify diurnal activity of aardvarks. We used a series of GLMMs to determine which independent variables influenced duration of active phase, proportion of diurnal activity, time of emergence and time of return to the burrows relative to time of sunset and sunrise, respectively. Independent variables included minimum 24-h body temperature, minimum 24-h air temperature, EVI, and photoperiod (time between sunrise and sunset) or time of sunset or time of sunrise. Again, we used each 24-h period as a sampling unit in the GLMMs, and added a first order autocorrelation structure to account for temporal autocorrelation. We confirmed VIF <2 for all independent variables in each model and aardvark identity was included as random factor. For time of emergence and return, camera trap data supplemented the dataset on days when no logger data were recorded (May to September 2015).

We used Python 2.7.13 (Python Software Foundation, United States) for the determination of daily time of emergence and time of return of aardvarks from their burrows. We used R statistical computing environment (R Core Team, 2018) with the package lme4 (Bates et al., 2015) to perform the GLMM analysis, and Excel 2016 (Microsoft Corporation, United States), Python 2.7.13 and Prism 5 (GraphPad Software, Inc., United States) to produce graphs. We present data as mean ± SD, and considered $P < 0.05$ to be statistically significant.

RESULTS

Environmental Conditions

Air temperatures at Tswalu varied seasonally, but interannual differences were small (**Figure 1A**), with annual summer air temperature maxima ranging between 37.9 and 39.9°C, whereas annual winter minima ranged between −1.5 and −4.4°C. In contrast, the amount and timing of rainfall showed high intra- and inter-annual variability, which in turn influenced vegetation greenness (**Figure 1B**). Total annual rainfall over 33 years, including 30 years before the study commenced, was highly variable (1982–2015: median 228 mm, 1st and 3rd quartile 178 and 270 mm, range 138–392 mm). In the year before our study started (July 2011–June 2012), rainfall was ∼340 mm, i.e., within the higher quartile range, and resulted in high vegetation productivity during that summer (∼85% of the maximum during our study) and the following winter. In year 1 of the study (July 2012–June 2013), rainfall was 196 mm, i.e., between the lower quartile range and the median, and no rain occurred during the hottest months of the summer. Vegetation productivity during the summer 2012–2013 and the following winter 2013 (termed "drought summer" and "drought winter") was less than ∼54% of the maximum EVI during our study. In year 2 (July 2013 to June 2014), rainfall was 461 mm, within the higher quartile range, and spread over 4 months. The high rainfall of the 2013–2014 summer resulted in the maximum vegetation productivity (EVI = 0.26) which only slowly declined until July 2014 even though rainfall virtually ceased after March. In year 3 (July 2014 to June 2015), rainfall was 172 mm, i.e., within the lower quartile range of the 33-year period, resulting in a slightly lower vegetation productivity that prevailed until year 4 (July to September 2015, when the study ended). Although the summer of year 3 (notably February 2015) was dry and EVI low, the

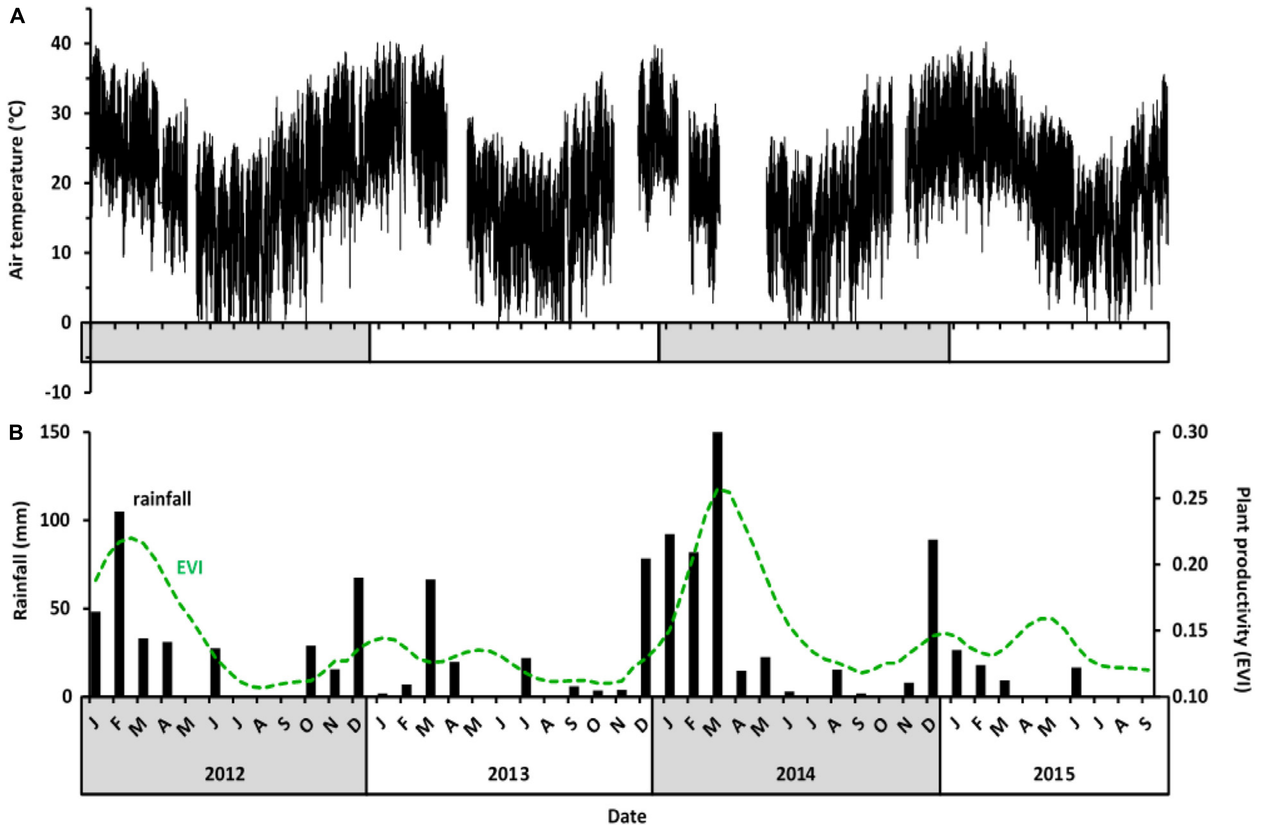

FIGURE 1 | Climate variables at Tswalu from July 2012 to September 2015. **(A)** Air temperature (°C) at 30-min-intervals. Temperature data were not available for periods in February 2013, November 2013, January 2014, and April 2014 because of weather station failure. **(B)** Monthly rainfall (black bars) and plant productivity presented as enhanced vegetation index (EVI; dotted green line) for the Gordonia duneveld vegetation at Tswalu.

rainfall was not delayed as long as it was during the summer of year 1, so the reduction in EVI was not as drastic, declining to ~62% of the maximum.

Patterns of Aardvark Body Temperatures

Body temperatures of study aardvarks ranged from 24.7 to 38.8°C (**Supplementary Table S5**). The greatest amplitude of 24-h body temperature rhythm was 11.7°C, recorded in aardvark *A11* on 30 July 2013, when its body temperature ranged from 26.1 to 37.8°C within eight hours. The recordings at five-minute intervals of body temperature of two representative aardvarks (*A06* and *A08*) throughout the study period are shown in **Figure 2A**. Periods of higher than usual variability in the amplitude of 24-h body temperature rhythm were evident in late summer 2012–2013 (notably March) and in winter 2013 (notably July to September). Similar patterns were observed in the mean, minimum, and maximum 24-h body temperatures of all study aardvarks (**Figure 2B**), with periods of the highest variability coinciding with aardvark deaths (**Figure 2B**, black arrows). Body temperature patterns of those aardvarks that survived the summer drought and the subsequent 2013 winter recovered to stable values with low variability of mean 24-h body temperature during the spring months, and most aardvarks maintained that state from October 2013 onward until the end of the study in September 2015. An exception was one aardvark that displayed pronounced variability in body temperature during the dry summer in February 2015, and again in winter 2015, before it died in late July 2015.

The GLMM results indicate that minimum 24-h body temperature of aardvarks was associated positively with minimum 24-h air temperature, photoperiod and with EVI (an index for food availability for aardvark prey; **Table 1A**). Maximum 24-h body temperature of aardvarks was associated positively with photoperiod and negatively with maximum 24-h air temperature, but not associated with EVI (**Table 1B**). Though significant, the influence of minimum and maximum air temperature on body temperature was trivial ($\beta < 0.01$). The amplitude of the 24-h body temperature rhythm was associated negatively with photoperiod and with EVI, but not associated with the range of 24-h air temperature (**Table 1C**), indicating that days with large fluctuations in air temperature were not associated with large fluctuations in body temperatures.

Activity Patterns

Locomotor activity of aardvarks over 24-h periods was biphasic, with activity typically high in the scotophase and low in the photophase, but with brief inactive bouts during the active phase, and brief active bouts during the inactive phases (as

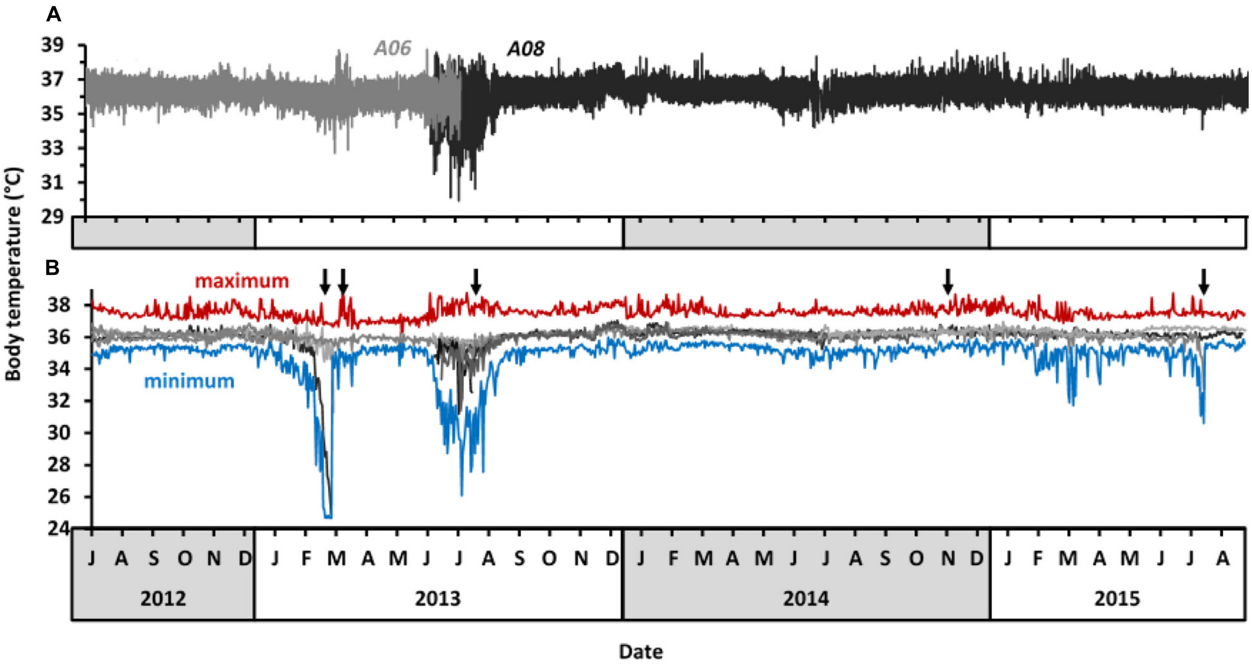

FIGURE 2 | Body temperature of study aardvarks at Tswalu between July 2012 and September 2015. **(A)** 5-min body temperature records of two representative study aardvarks at Tswalu (*A06* and *A08*). **(B)** Overview over the 24-h body temperature profile of all study aardvarks. Grey lines represent mean 24-h body temperature of different individuals, with the individuals from panel **(B)** in the same colours as in panel **(A)**. The upper red and lower blue lines display the absolute maximum and absolute minimum body temperature, respectively, of all study aardvarks within each 24-h period to indicate the total range of body temperatures reached in aardvarks. Black arrows indicate when study aardvarks died.

TABLE 1 | GLMM results showing effects of photoperiod (hours between sunrise and sunset), minimum or maximum or range of air temperature (°C), and EVI of Gordonia duneveld vegetation (an index of vegetation greenness and thus food availability for aardvark prey) at Tswalu on **(A)** minimum, **(B)** maximum, and **(C)** amplitude of the body temperature rhythm (°C) for each 24-h period between July 2012 and September 2015.

Body temperature (*N* = 3749)	**Variable**	**β ± SE**	***t***	***P***
(A) 24-h minimum	Minimum air temperature	0.007 ± 0.002	3.4	*<0.001*
	EVI	3.9 ± 2.0	2.0	*<0.05*
	Photoperiod	5.4 ± 1.4	3.7	*<0.001*
	intercept	31.5 ± 0.8	39.5	*<0.001*
(B) 24-h maximum	Maximum air temperature	− 0.004 ± 0.002	−2.2	*<0.001*
	EVI	0.9 ± 1.2	0.7	0.47
	Photoperiod	1.7 ± 0.9	2.0	*0.04*
	Intercept	36.4 ± 0.5	80.7	0.48
(C) 24-h amplitude	Range of air temperature	0.004 ± 0.003	1.7	0.10
	EVI	− 6.9 ± 1.2	−6.0	*<0.001*
	Photoperiod	− 3.8 ± 0.8	−5.0	*<0.001*
	Intercept	5.1 ± 0.4	10.7	*<0.001*

Aardvark identity was included as a random factor (n = 10 aardvarks). Significant P-values are italicised for ease of reference.

shown for a representative aardvark *A08*, **Figure 3**). Patterns of activity of aardvarks varied intra- and inter-annually (**Figures 3**, **4**). Aardvarks were generally active for ∼8 h, but shortened their active phases to ∼6 h during winters (**Figure 4C**). Late in our study period, aardvarks were exclusively nocturnal, typically emerging at sunset and returning to their burrows at sunrise (**Figure 4A**). However, early in our study period, there was substantial diurnal activity, accompanied by early returns to the burrows (**Figure 4A**). Occasionally aardvarks emerged as early as 8 hours before sunset and occasionally they returned to their burrows before sunset, so they had entirely diurnal activity periods. In spite of the variation in times of emergence and return, the duration of the activity period was remarkably constant, except during the winter months, when early returns to the burrows led to curtailed activity periods (**Figure 4C**). Shifts of the times of the start and end of activity were not synchronised, resulting in a shortening of the active phase coinciding with a shift to diurnal activity (**Figure 4**).

There was no particular air temperature at which aardvarks emerged from their burrows or returned to them (**Figure 4B**). The range of air temperatures at which aardvarks emerged (−3.2 to 36.9°C) or returned (−2.4 to 39.4°C) to burrows varied seasonally, and was only slightly narrower than the full range of air temperatures experienced at Tswalu (**Figure 4B**). Air temperatures usually were higher at the time of emergence than

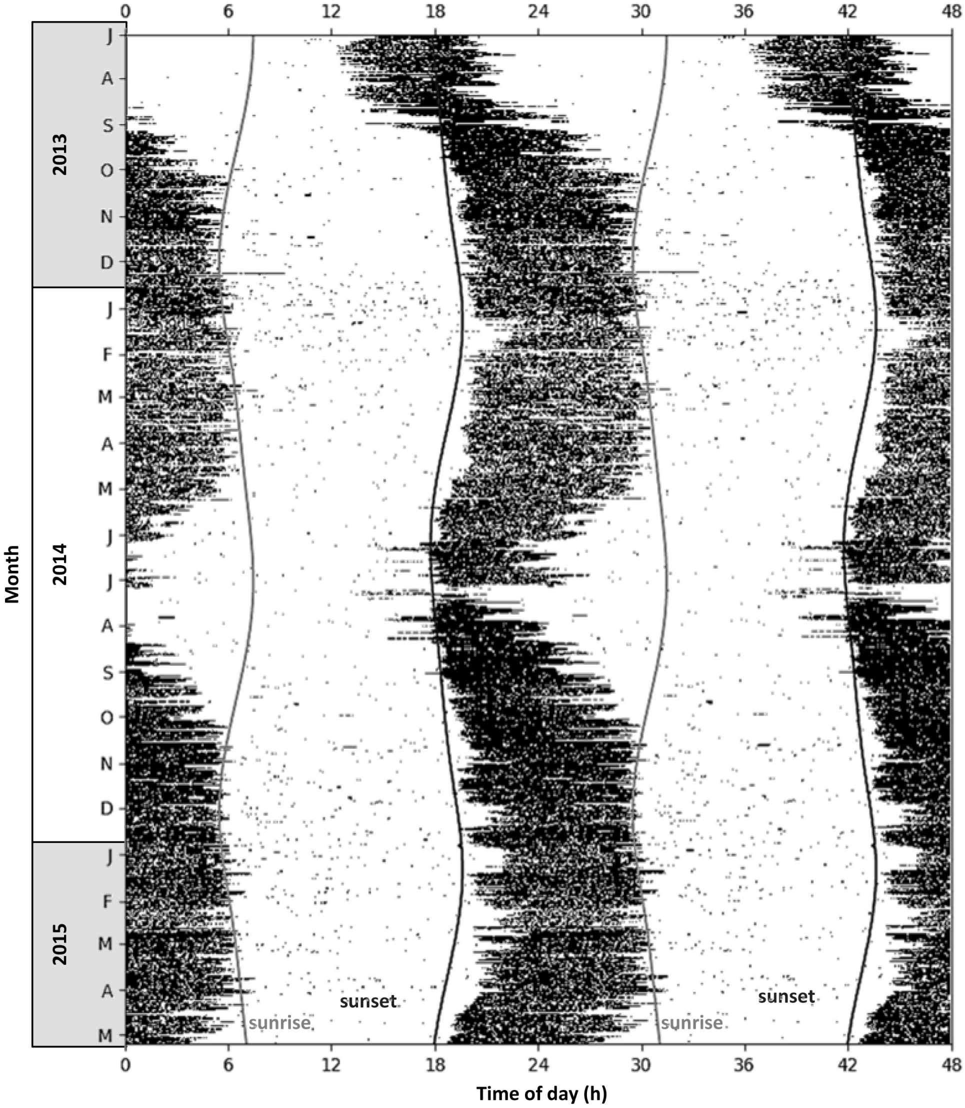

FIGURE 3 | Actogram showing locomotor activity of a single representative aardvark (*A08*) at Tswalu, free-ranging in its natural environment, for the period July 2013 to May 2015 (*N* = 675 consecutive days). Days are stacked vertically, data are displayed in 5-min intervals. Black bars represent periods when the aardvark was active (≥5% of maximum activity); white areas represent periods when the aardvark was inactive (<5% of maximum activity); dark grey line is the time of sunset; light grey line is the time of sunrise. For better visualisation of seasonal shifts in nychthemeral rhythms, activity is plotted over 48 h, with two consecutive 24-h periods displayed beside each other.

at the time of return, except from January to March 2013, when aardvarks had increased diurnal activity.

The GLMM analyses of variables affecting aardvark activity patterns indicate that the daily duration of the active phase of aardvarks was associated strongly and positively with photoperiod and EVI, and weakly but positively with minimum 24-h body temperature and minimum 24-h air temperature (**Table 2A**). The proportion of activity that took place diurnally was associated inversely with minimum 24-h body temperature, minimum 24-h air temperature, photoperiod and EVI (**Table 2B**). Time of aardvark emergence from a burrow relative to sunset was associated positively and strongly with minimum 24-h body temperature and EVI, but not associated with time of sunset or minimum 24-h air temperature (**Table 2C**). Time of return to a burrow relative to sunrise was associated strongly and positively with EVI and minimum 24-h body temperature, strongly and negatively with time of sunrise, and positively but weakly with minimum 24-h air temperature (**Table 2D**).

Patterns of 24-h Body Temperature and Activity

To visualise how low resource availability during the drought influenced body temperature and activity of our aardvarks, we graphed (**Figure 5**) aardvark activity and body temperature, as a

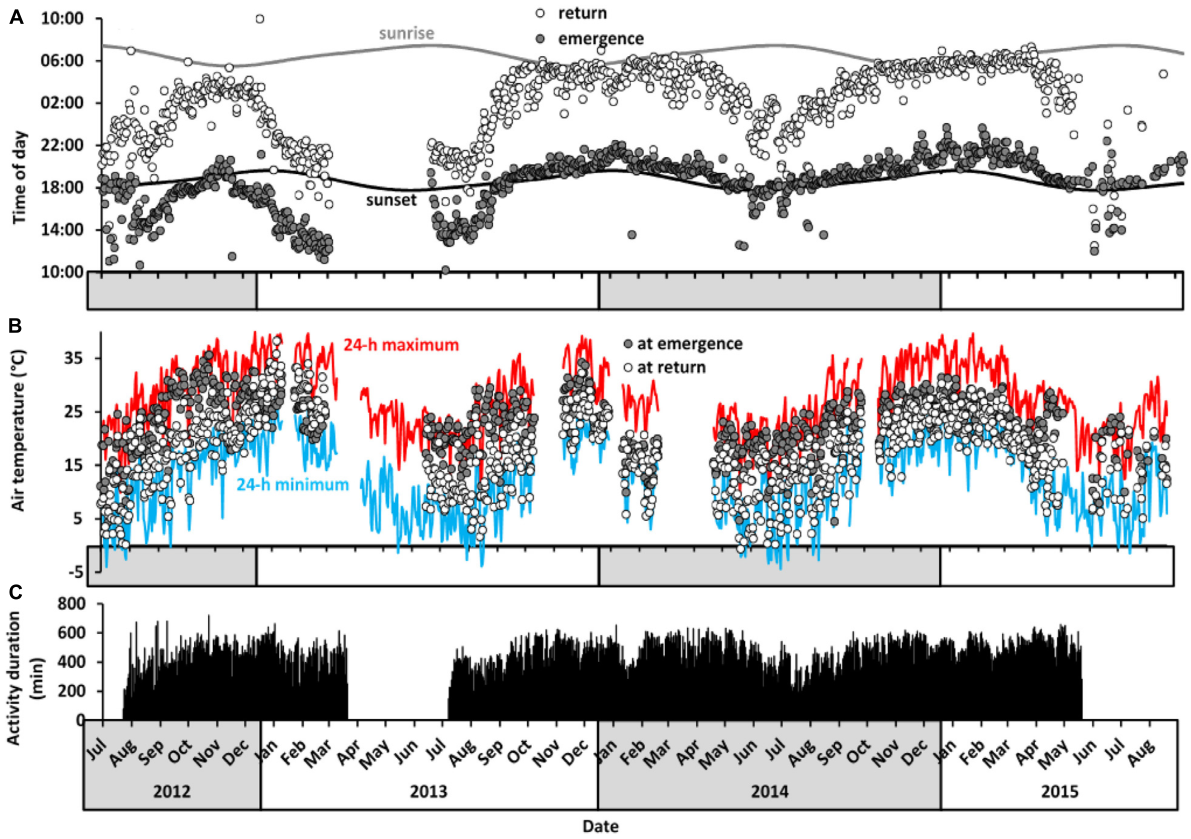

FIGURE 4 | Long-term activity patterns of aardvarks at Tswalu between July 2012 and September 2015. **(A)** Daily time of emergence and return to burrow of all study aardvarks in relation to times of sunrise and sunset. Values are averages for all aardvarks (*n* = 1 to 5 aardvarks) per day (*N* = 1,032 days). **(B)** Air temperature at time of emergence from (grey circles) and return to (white circles) the burrows, averaged across all aardvarks per day. The blue line represents minimum 24-h air temperature and the red line represents maximum 24-h air temperature. Temperature data were not available for periods in February 2013, November 2013, January 2014, and April 2014 because of weather station failure. **(C)** Duration of 24-h activity averaged across study aardvarks. Activity logger data were not available between March and July 2013 and after June 2015 because of failure of data loggers and deaths of several study aardvarks (see **Supplementary Table S1** for details).

function of time of day, for a winter and summer month, during a drought year (rainfall in the lowest quartile of a 33-year record) and during a non-drought year (rainfall in the highest quartile of a 33-year record). In the absence of drought, body temperature assumed a bimodal rhythm, with elevated temperatures at night, coinciding with aardvarks' nocturnal activity, and a lower plateau (February 2014, summer, **Figure 5C**) or depression with slight decline (August 2014, winter, **Figure 5D**) coinciding with daily inactive phases inside the burrows. Over the winter month, which followed a non-drought summer, aardvarks shifted their activity to slightly earlier times of day than in summer, becoming partly diurnal, and the nocturnal peak in body temperature occurred somewhat earlier (**Figure 5D**).

Those body temperature profiles were substantially distorted during a drought. The profiles of body temperature no longer were bimodal but more cosinor. The 24-h maxima of body temperature differed little from those in the absence of drought, but in the winter month (August 2013, **Figure 5B**), the 24-h maximum occurred before sunset, coinciding with a shift in activity pattern as aardvarks emerged earlier, with activity starting to increase during the late morning, and peaking during the early evening. During the drought summer month (February 2013, **Figure 5A**), the two aardvarks for which we retrieved activity data had different activity patterns; one became diurnal and the other remained largely nocturnal. The average body temperature of these two, plus two other aardvarks for which we retrieved body temperature data but not activity data during this particular month, continued to peak at night, but now after a gradual increase beginning soon after sunrise. The stable body temperatures that occurred during the day in the absence of a drought disappeared, presumably because some aardvarks were not in the burrow, and body temperature dipped to a nadir occurring a few hours after sunrise. In the drought winter month (**Figure 5B**), that nadir, averaged for six aardvarks for which we retrieved body temperature data, was below 34°C. Variability in body temperature between aardvarks, as indicated by the standard deviation, was much higher during the drought winter month than in the non-drought winter month, when aardvarks had remarkably similar body temperatures. In the summer months, drought had little influence on that variability.

TABLE 2 | GLMM results showing effects of minimum 24-h body temperature (°C), minimum 24-h air temperature (°C), photoperiod (hours between sunrise and sunset) or time of sunset or time of sunrise, and EVI of Gordonia duneveld vegetation (an index of vegetation greenness and thus food availability for aardvark prey) at Tswalu, on **(A)** duration of the active phase (hours), **(B)** proportion of the daily active phase spent active during daylight hours, **(C)** time of emergence from burrows relative to sunset, and **(D)** time of return to burrows relative to sunrise for each 24-h period between July 2012 and September 2015.

Activity	Variable	β ± SE	*t*-value	*P*
(A) Duration of the active phase (*N* = 2,254)	Minimum body temperature	0.008 ± 0.002	3.3	*< 0.001*
	Minimum air temperature	0.002 ± <0.001	5.4	*< 0.001*
	Photoperiod	0.19 ± 0.07	2.8	*0.005*
	EVI	0.27 ± 0.07	3.7	*< 0.001*
	Intercept	− 0.11 ± 0.07	− 1.4	0.15
(B) Proportion of the active phase diurnal (*N* = 2,254)	Minimum body temperature	− 5.2 ± 0.6	− 9.0	*< 0.001*
	Minimum air temperature	− 0.18 ± 0.08	− 2.2	*0.03*
	Photoperiod	− 41.8 ± 19.9	− 2.0	*0.04*
	EVI	− 63.7 ± 25.2	− 2.5	*0.01*
	Intercept	239.0 ± 22.4	10.7	*< 0.001*
(C) Time of emergence (*N* = 2,243)	Minimum body temperature	0.39 ± 0.07	5.7	*< 0.001*
	Minimum air temperature	0.014 ± 0.009	1.5	0.12
	Time of sunset	4.3 ± 5.1	0.8	0.41
	EVI	7.6 ± 3.4	2.2	0.03
	Intercept	− 19.4 ± 4.4	− 4.4	*< 0.001*
(D) Time of return (*N* = 2,243)	Minimum body temperature	0.31 ± 0.08	3.8	*< 0.001*
	Minimum air temperature	0.05 ± 0.01	4.8	*< 0.001*
	Time of sunrise	− 77.0 ± 5.5	− 14.2	*< 0.001*
	EVI	22.3 ± 3.8	6.0	*< 0.001*
	Intercept	0.97 ± 3.51	0.3	0.78

No activity data were available from March to June 2013. For time of emergence and return, camera trap data were included in the dataset on days on which no biologger data were recorded (May to September 2015). Aardvark identity was included as a random factor (n = 7 aardvarks). Significant P-values are italicised for ease of reference.

The mean amplitude of the 24-h rhythm of aardvark body temperature during a non-drought summer month (1.8 ± 0.3°C) increased during a drought summer to 2.3 ± 0.4°C, which was similar to that evident in a non-drought winter month (2.1 ± 0.1°C). Mean amplitude of 24-h body temperature rhythm in a drought winter month (4.7 ± 1.3°C) was more than double the amplitude during the equivalent month in the absence of a drought.

DISCUSSION

Our long-term study over ∼3.5 years assessed intra-and inter-annual variability in aardvark body temperature and activity responses to fluctuations in environmental conditions in the semi-arid Kalahari and allowed us to elucidate differences between drought and non-drought years. During a drought year, vegetation was scarce. Aardvarks were partly diurnal during the drought summer as well as during much of the subsequent winter, and displayed minimum 24-h body temperatures as low as 24°C, resulting in exaggerated heterothermy. Several aardvarks died during the drought summer and the following winter after exhibiting large amplitudes of the 24-h body temperature rhythm and diurnal foraging activity. In comparison, during non-drought years, when above-average rainfall generated high vegetation productivity and thus likely high availability of aardvark prey (Weyer, 2018), aardvarks remained nocturnal throughout the summer and most of the winter. Rather than extending their foraging duration in an attempt to obtain more food, aardvarks shortened their foraging duration during the drought and winter, most notably during a winter following drought. Our data showed that, of the variables that we measured, the key driver of changes in body temperature and activity in our Kalahari aardvarks was EVI. In our GLMMs, EVI (a measure of vegetation greenness) was associated positively with minimum 24-h body temperature, and very strongly and inversely with amplitude of the 24-h body temperature rhythm, an index that we have proposed for the well-being of mammals (Hetem et al., 2016). EVI also was associated positively with the duration of the aardvarks' foraging activity and with the time of emergence and return to the burrows, which resulted in an increase in the proportion of diurnal activity that took place when vegetation was brown. Moreover, minimum 24-h body temperature was a relevant driver of aardvark activity, such that the lower their minimum 24-h body temperature was, the more likely aardvarks were to become diurnal, and to emerge from and return to their burrows earlier. We conclude that the aberrations that we observed in body temperature and activity patterns during droughts were mainly related to energy deficits, and the aardvarks behaved in a way that reduced the thermoregulatory cost of those energy deficits (see **Figure 6**). Similar declines in minimum 24-h body temperature have been observed in other large mammals that experienced an energy deficit (Hetem et al., 2016). For example, Arabian oryx (*Oryx leucoryx*) showed low minimum 24-h body temperatures throughout the dry, resource-scarce period, irrespective of ambient temperatures (Hetem et al., 2010).

It was not low EVI itself that resulted in energy deficits during droughts. Vegetation greenness provided a measure of resource availability because the aardvarks' primary prey item, the harvester termite *Hodotermes mossambicus* (Weyer, 2018), consumes grass (Nel and Hewitt, 1969; Symes and Woodborne, 2011). The higher the EVI, the more abundant or nutritious would have been the termites, and the greater the amount of dietary energy, and dietary water, available readily to the aardvarks. The aardvarks consequently lost body condition when EVI was low (Weyer, 2018). Loss of body fat would have compromised the capacity of the aardvarks to respond metabolically to cold. If they were to respond, aardvarks would have to do so behaviourally by retreating to warmer burrows and exploiting ambient warmth during the day. That they did by engaging in diurnal activity, and the lower their minimum 24-h body temperatures were, the greater was the proportion of

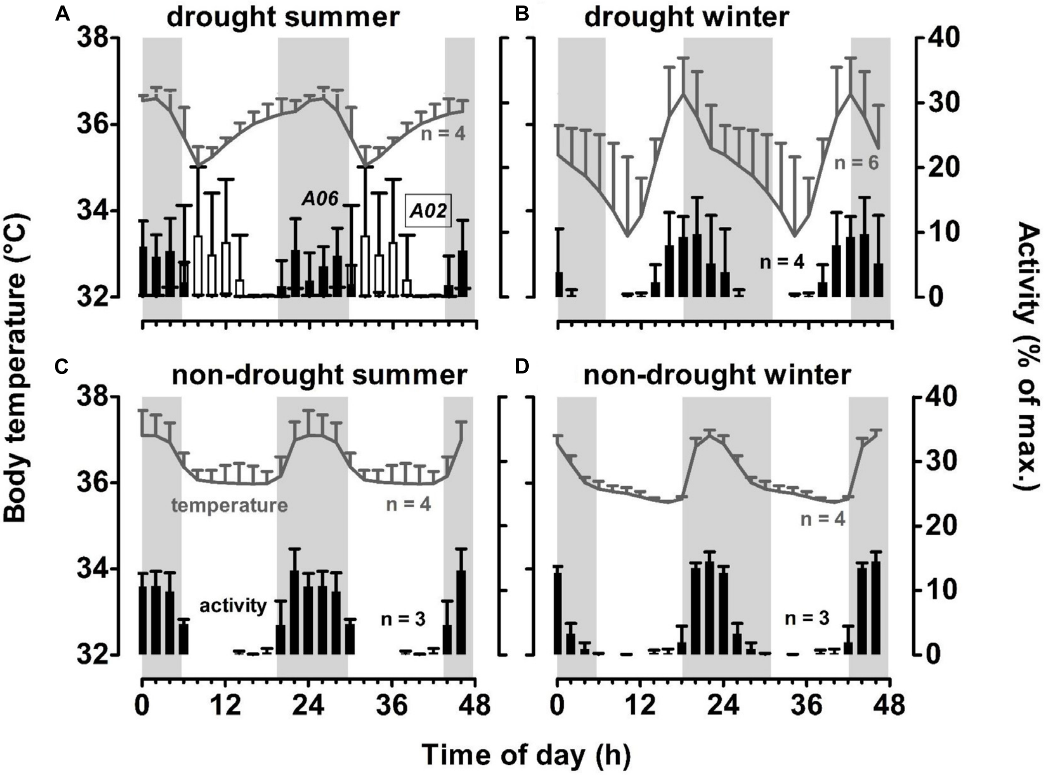

FIGURE 5 | Patterns of 24-h activity and body temperature of study aardvarks, double-plotted for better visualisation, during a month of a drought summer (February 2013, panel **(A)**, the following drought winter (August 2013, panel **(B)**, during a non-drought summer (February 2014, panel **(C)**, and the following non-drought winter (August 2014, panel **(D)** at Tswalu. Grey areas indicate night time. Error bars indicate SD between individuals. Note that on panel **(A)**, activity patterns of two aardvarks for which data were available were plotted individually to display the difference between an aardvark that survived the drought summer (*A06*, filled bars), and one that died in the following month (*A02*, empty bars). Mean minimum 24-h air temperatures during the winter months were similar (4.5°C in August 2013; 7.6°C in August 2014). Mean maximum 24-h air temperatures differed between the summer months, with the drought summer up to 10°C hotter than the non-drought summer month (36.5°C in February 2013; 26.0°C in February 2014.

their active phase that took place diurnally. Many aardvarks died at our study site at the end of the 2013 summer drought (Rey et al., 2017), with some aardvarks shifting activity entirely to the daylight hours in the weeks leading up to their deaths. Those deaths and failure of some of our biologging devices reduced our sample size and we did not have continuous measurements of body temperature and activity in the same individuals over the 3.5-year study, but we are confident that our GLMM analyses were robust. Indeed, these analyses have allowed us to present the longest and most comprehensive study on the ecophysiology of free-living aardvarks to date.

Aardvarks were affected adversely by reduced prey availability not only during the 2013 summer drought itself, but afterwards, because surviving aardvarks took a long time to improve their body condition (indicated by return of homeothermic body temperature patterns) after the drought winter. The single individual (*A06*, **Figure 2**) for which data are available for the autumn after the 2013 drought summer did not show diurnal activity and had only short bouts of heterothermy during the summer drought compared to other aardvarks, and showed a rapid return to stable thermoregulation following the onset of summer rains, but a second decline in minimum body temperature and longer-lasting heterothermy during the subsequent cold and dry winter. During this winter (2013), additional aardvarks died (including one of our study aardvarks) and no study aardvark maintained stable body temperature at night (mean amplitude of the 24-h body temperature rhythm during August 2013 was 4.7 ± 1.3°C; absolute range 24.7 to 38.8°C). The 11.7°C amplitude of the 24-h body temperature rhythm in a surviving individual during that winter is the largest recorded in any large, free-living mammal to date, larger than that previously reported for aardvarks during the summer drought (8.6°C; Rey et al., 2017) or for Arabian oryx in the harsh Arabian Desert (7.7°C; Hetem et al., 2010). The variable minimum 24-h body temperatures of our aardvarks are incongruent with the controlled downregulation of body temperature as a result of a reduction of the hypothalamic set-point, as in torpor (Heldmaier and Ruf, 1992). Rather than a regulated response, the loss of body condition and high mortality rate associated with lowered 24-h minimum body temperatures of our aardvarks imply that the animals were experiencing an energy deficit. Like our aardvarks during non-drought conditions, free-living aardvarks during winter and summer in the Karoo, South Africa displayed a bimodal 24-h rhythm of body temperature with lower body

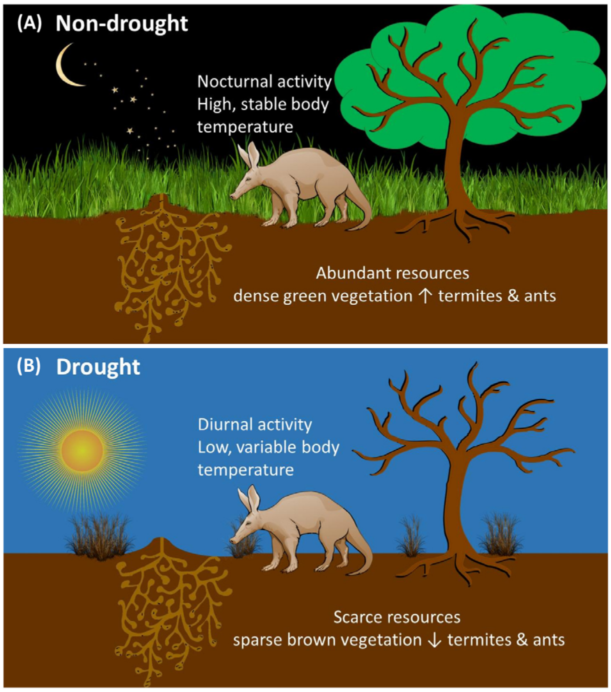

FIGURE 6 | Pictogram illustrating the interplay of different factors influencing aardvark body temperature and activity **(A)** during a non-drought period with green grass and plenty of prey termites and ants available, aardvarks are active at night and maintain high and stable body temperature rhythms; **(B)** during a drought period with sparse brown grass and few prey insects deep in burrows aardvarks are active during the daytime and display low and variable body temperature rhythms.

temperature during the day when the aardvarks were inactive inside their burrows, and higher body temperatures at night when they were foraging actively (Taylor and Skinner, 2004). Taylor and Skinner (2004) suggested that the endogenous heat produced by intense digging might restrict aardvark foraging to the cooler night-time during hot periods whereas sensitivity to cold might force aardvarks to shift their foraging activity earlier; they claimed that aardvarks avoided air temperatures below 2°C. Our data do not support those suggestions. On occasion, our aardvarks still were active at air temperatures below freezing (compare panels **A** and **B** of **Figure 4**), and we observed them engaged in digging activity during the heat of the day in summer.

When aardvarks had low minimum 24-h body temperatures, they increased the time they spent active during daylight hours but decreased their total foraging duration. Optimal foraging theory proposes that an animal should maximise energy intake while taking into account all potential costs and benefits of foraging (Krebs, 1980; Pyke, 1984), such that the animal would continue to forage as long as energetic benefits exceed the likely costs of foraging, including predation risk and energy costs (Lima and Dill, 1990). When resources become limited, an animal might either increase the duration or intensity of its foraging activities to locate sparse resources (Therrien et al., 2008; Clutton-Brock et al., 2009; Podolski et al., 2013), or decrease activity to conserve energy (O'Donnell, 2000; Christian and Geiser, 2007). During a drought summer and resource-limited winters, when prey availability was reduced, our aardvarks did not compensate by foraging for longer, likely to avoid energetic costs incurred by foraging, and consequently lost body condition, sometimes lethally.

The activity pattern of our aardvarks in the Kalahari during non-drought years, when it was not dominated by the need to survive starvation, resembled that of aardvarks in the Karoo (Van Aarde et al., 1992; Taylor, 1998), in that when days were long in summer (and therefore the nights were short), the aardvarks counterintuitively were active for longer (~8 h per day), and almost exclusively nocturnal. During short days in winter, both our aardvark and those in the Karoo (Taylor, 1998) reduced the duration of activity (~6 h per day) and aardvarks emerged from and returned to their burrows earlier in the day, particularly when resource availability (as indexed by EVI) was low.

In terms of aardvarks timing their activity according to their prey availability, optimal foraging theory predicts that a species would coordinate the timing of its foraging activities with the times of the day at which their prey is most accessible, to maximise energy intake. Harvester termites, a key prey item of Kalahari aardvarks (Weyer, 2018), occur in deep (up to 7 m) underground hives (Hartwig, 1965). To harvest grass (Nel and Hewitt, 1969; Symes and Woodborne, 2011), these termites must undergo daily vertical migrations from the deep hive area and move to the upper, warmer soil levels during the day, to warm up in anticipation of their daily foraging. Thus, in winter, termites might aggregate near the surface several hours before emerging to forage during the daytime, which might explain a shift in aardvark foraging to earlier times in winter. In summer, the termites would be expected to migrate toward the upper levels of the soil in the evening, in anticipation of their night-time foraging, which would match the nocturnal activity of the aardvarks in summer. However, aardvarks at Tswalu became mostly diurnal during drought winters but less so during non-drought winters, thus an adaptation of their active phase to the timing of termite activity was unlikely to have been the explanation for the seasonal shift in aardvark activity. Similarly, in the Karoo, aardvark activity was independent of prey activity as the aardvarks were nocturnal but their predominant prey ants *Anoplolepis custodiens* (Taylor et al., 2002) were predominantly diurnal (Lindsey and Skinner, 2001). Moreover, harvester termites also have been reported to forage diurnally even at high temperatures (Nel, 1970), substantiating the argument that aardvark activity is independent of that of prey activity. That our aardvarks could forage at night or in the day implies that termites were available to them at all times.

The main factor that influenced the proportion of activity that was diurnal in our aardvarks was their minimum 24-h body temperature, proposed as an index of nutritional state (Rey et al., 2017). Our aardvarks switched to more-diurnal activity after they no longer had sufficient energy to maintain body temperature at night. During periods of negative energy balance, other nocturnal mammals also shift their activity to the daytime, both in the laboratory (Hut et al., 2011; Van der Vinne et al., 2014, 2015) and in the wild (Lockard, 1978; Boal and Giovanni, 2007). The shift to diurnality in response to an energy deficit is more pronounced under cold conditions (Van der Vinne et al., 2014), when metabolic costs associated with thermoregulation are highest. Such plasticity of the mammalian circadian system is thought to be an adaptive mechanism that allows nocturnal mammals to conserve their energy (up to 10% in some species, Van der Vinne et al., 2015), thereby enhancing their survival (Van der Vinne et al., 2019). However, if the shift to diurnality occurs when ambient temperatures are high (Van der Vinne et al., 2014), as it did in our aardvark during the summer drought, it would increase their exposure to heat.

The suite of adaptive responses available to aardvarks that has been sufficient to ensure their survival in the past may not be sufficient in the future. In semi-arid zones with summer rainfall, such as the Kalahari, availability of resources is dictated primarily by the timing and amount of rainfall (Noy-Meir, 1973; Hawkins et al., 2003), and delayed onset of annual rains, as projected in the Kalahari under climate change (Shongwe et al., 2009), results in low vegetation productivity at a time when conditions are getting hotter and drier. Summer droughts combined with heat waves, as observed in the summer of 2013, will become increasingly frequent in southern Africa as a result of climate change (Niang et al., 2014; Phaduli, 2018), as has already been evident during the past decade (Jordaan, 2012; Agri-SA, 2019; Ngqakamba, 2019). Such climatic changes and consequent changes in resource availability might severely impact the persistence of aardvarks if their physiological and behavioural capacity to adapt is exceeded. Extirpation of aardvarks, key ecosystem engineers that provide thermal refugia to dozens of vertebrates (Whittington-Jones et al., 2011), might have severe cascading effects for biodiversity in the Kalahari ecosystem.

DATA AVAILABILITY STATEMENT

The data sets analysed for this study are available on request to the corresponding author.

ETHICS STATEMENT

All procedures were approved by the Department of Environment and Nature Conservation of South Africa (Northern Cape Province Government, permits no. FAUNA 1000/2013, 1000/2/2013, and 1001/2013) and the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance certificate no. AESC 2011/10/04 and AESC 2013/29/05).

AUTHOR CONTRIBUTIONS

All authors contributed to study design, field work and data interpretation, edited the manuscript, approved the final version of the manuscript, and agreed to be held accountable for the content therein. AH and LM carried out surgical procedures. NW and RH analysed the data. NW drafted the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00637/full#supplementary-material

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REVIEW
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Integrating Mortality Risk and the Adaptiveness of Hibernation

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Low mortality rate is often associated with slow life history, and so far, has mainly been assessed through examinations of specific adaptations and lifestyles that limit mortality risk. However, the organization of activity time budgets also needs to be considered, since some activities and the time afforded for performing them may expose animals to higher mortality risks such as increased predation and/or increased metabolic stress. We examined the extent of activity time budgets contribution to explaining variation in life history traits in mammals. We specifically focused on hibernating species because of their marked seasonal cycle of activity/inactivity associated with very different mortality risks. Hibernation is considered a seasonal adaptation to prolonged periods of food shortage and cold. This inactivity period may also reduce both extrinsic and intrinsic mortality risks, by decreasing exposure to predators and drastically reducing metabolic rate. In turn, reduction in mortality may explain why hibernators have slower life history traits than non-hibernators of the same size. Using phylogenetically controlled models, we tested the hypothesis that longevity was positively correlated with the hibernation season duration (the time spent between immergence and emergence from the hibernaculum or den) across 82 different mammalian species. We found that longevity increased significantly with hibernation season duration, an effect that was particularly strong in small hibernators (<1.5 kg) especially for bats. These results confirm that hibernation not only allows mammals to survive periods of energy scarcity, but further suggest that activity time budgets may be selected to reduce mortality risks according to life history pace.

Keywords: activity patterns, hibernation, life history, temporal organization of activity, time and energy allocations, trade-off, longevity, mortality risk

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INTRODUCTION

Energy is the fundamental requirement for life. Its acquisition, storage, and metabolic use shape the diversity of lifestyles in all living organisms (Brown et al., 2004). Because energy availability to organisms is limited under natural conditions, in terms of its acquisition in time and space, as well as its quantity and quality, organisms have to simultaneously maximize investments into all biological functions, and to compromise the allocation of metabolic energy among competing demands (Lack, 1966; Williams, 1966; Hirshfield and Tinkle, 1975; Reznick, 1985). For example, demographic characteristics may affect energy investments among biological traits such as growth, reproduction, and somatic maintenance. These trade-offs have led to the evolution of specific life history strategies (Stearns, 1992).

Attributes of life histories generally scale with body size such that large animal species usually take longer to develop and mature, have lower annual reproductive rates, and live longer compared to small species (Blueweiss et al., 1978; Speakman, 2005). However, life history variations that are specific to a particular body size are also observed along a fast-slow continuum (Oli, 2004; Bielby et al., 2007; Dobson and Oli, 2007; Jones et al., 2008; Jeschke and Kokko, 2009). For a given body size, most species trade off investments between reproduction and self-maintenance. For example, a species with fast life history strategy will exhibit faster growth, earlier reproduction, larger annual reproductive investment, and reduced maximum life span compared to a species with a slow life history strategy, which will typically promote self-maintenance and survival over reproduction (but see Bielby et al., 2007; Jeschke and Kokko, 2009).

The evolution of the fast-slow continuum in life history strategies appears to be contingent upon individual mortality risk (Promislow and Harvey, 1990; Martin, 2015; Healy et al., 2019). Individual mortality can be due to either intrinsic (wear-and-tear of the body) or extrinsic factors, such as predation, disease, or environmental hazards. Species with slow life histories typically exhibit adaptations that limit both sources of mortality (Holmes and Austad, 1994; Wilkinson and South, 2002; Blanco and Sherman, 2005; Sibly and Brown, 2007; Munshi-South and Wilkinson, 2010; Shattuck and Williams, 2010; Turbill et al., 2011; Lewis et al., 2013; Healy et al., 2014; Healy, 2015; MacRae et al., 2015; Wu and Storey, 2016). Besides these molecular (such as oxidative stress tolerance), physiological/anatomical (such as chemical protection, horns and antlers) or lifestyle (such as arboreality) adaptations that reduce mortality risk, the organization of activity time budgets should be particularly important in shaping the variety of life histories observed in the wild. Mortality rates may change depending on the time allocated to each activity, resulting in trade-offs for which both time and energy can be optimized. For instance, activities that contribute the most to reproductive success are often energy-intensive (Alonso-Alvarez et al., 2004) and associated with higher risks of extrinsic mortality (Magnhagen, 1991). However, the temporal dimension of energy allocation trade-offs in relation to the evolution of life history strategies has been little examined (see Healy et al., 2014).

Here, we first examined the extent to which patterns of relative activity and inactivity might explain variation in life history traits in mammals. We specifically focused on hibernating species because of their marked seasonal cycle of activity/inactivity, which is associated with very different risks of mortality. From an intrinsic perspective, hibernation is a period of metabolic depression where energy requirements are reduced to minimal levels compared to the active season (Ruf and Geiser, 2015). It has been suggested that energy restriction slows down the aging process (Walford and Spindler, 1997; Masoro, 2006) and is associated with enhancement of somatic maintenance (Shanley and Kirkwood, 2000). From an extrinsic perspective, even if mortality during hibernation occurs, hibernating mammals are usually hidden in burrows or shelters, which may reduce risks of predation, infections or injuries for several continuous months. As a result, hibernating species generally exhibit lower rates of mortality than similar-sized non-hibernating species during part of the year, resulting in slower life history strategies (Wilkinson and South, 2002; Turbill et al., 2011). However, previous studies linking hibernation to longevity considered hibernation as a binary trait (if animals hibernate or not), rather than a continuous adaptive response (hibernation season duration) allowing animals to restrict their period of activity during parts of the year. Yet, early data from captivity highlight a positive correlation between longevity and hibernation duration in Turkish hamsters (*Mesocricetus brandti*) (Lyman et al., 1981). In addition, a strong negative effect of mean annual temperature on hibernation season duration and annual survival rate, which is highly correlated with longevity (Turbill et al., 2011), has been shown among populations of hibernating rodent species (Turbill and Prior, 2016). This raises the question of whether, across hibernating mammals, the time spent being inactive (hibernation season duration) influences maximal longevity, a key feature characteristic of fast and slow life history strategies.

In the present study, we tested for a positive association between the hibernation season duration and longevity across 82 mammalian species. For this examination, we tested for effect of body size on longevity while controlling statistically for phylogeny. If indeed hibernation season duration is part of a strategy that minimizes mortality, we predicted that a longer hibernation season duration should be positively associated with species maximum longevity, especially in small mammals (<1.5 kg) that exhibit greater longevity than non-hibernators of the same size (Turbill et al., 2011). In addition, if metabolic reduction during hibernation slows aging (Lyman et al., 1981; Turbill et al., 2013; Wu and Storey, 2016), we predicted that the effect of hibernation season duration on longevity should increase with the percentage of metabolic reduction during hibernation compared to euthermia.

MATERIALS AND METHODS

Review Criteria

We conducted the review using the search engine Google Scholar[1] and considered articles up to and including December 2019. We based our survey on the hibernating species listed in Turbill et al. (2011) and Ruf and Geiser (2015), and further identified nine other species, mainly ground squirrels and bats, not mentioned in any of the lists. All the 152 hibernators that were examined in this process are summarized in **Supplementary Material** (see **Supplementary Table S1**). We excluded species for which hibernation was restricted to only a few populations or under specific conditions, namely two species, the black-tailed prairie dog (*Cynomys ludovicianus*;

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[1] https://scholar.google.com

Gummer, 2005; Lehmer et al., 2006) and the polar bear (*Ursus maritimus*; Amstrup and DeMaster, 2003). We did not include non-seasonal hibernating species capable of entering multi-day torpors at any time of the year. Indeed, for such cases, it is difficult to accurately measure the time spent in hibernation over a year, which may also vary considerably among individuals and between years. Thus, we excluded hibernating elephant shrew species (*Elephantulus* sp.) and hibernating marsupials with the exception of the mountain pygmy possum (*Burramys parvus*), reported to be a seasonal hibernator (Lovegrove et al., 2001; Geiser and Körtner, 2010).

Longevity and Body Mass Data

Data on maximum longevity, hereafter referred as longevity, and average body mass for the list of hibernating species previously identified were mainly obtained from the AnAge data base (The Animal Aging and Longevity Database[2]; Human Ageing and Genomic Resources; Magalhães and Costa, 2009), and complemented these data with information from the PanTHERIA data base (Ecological Society of America[3]; Jones et al., 2009) from two reviews on mammalian longevity (Heppell et al., 2000; Wilkinson and South, 2002) and from a specific search in Google Scholar combining the following terms: "longevity" OR "life history" AND scientific or common names of species. For this specific search in Google Scholar, we considered both old and new nomenclatures for ground squirrel species, and only selected long-term field studies since they provide a good estimate of maximum longevity. Moreover, we specifically investigated the source of the data (captive vs. wild) and only retained longevity data where the source was available, to control for captivity effects on longevity (see **Supplementary Table S2** for references).

We determined the arboreality lifestyle of the species in order to statistically control (see below section "Statistics") for higher longevity (Kamilar et al., 2010; Shattuck and Williams, 2010; Healy et al., 2014). For this, we conducted a review of peer-reviewed scientific journal Mammalian Species[4] and used Google Scholar. The search criteria were based on combining the following terms: "arboreal" OR "semi-arboreal" OR "climb tree" AND scientific or common names of species. We completed our search by examining the mammalian lifestyle databases of the following articles (Healy et al., 2014; Hidasi-Neto et al., 2015). Our arboreality factor included 16 arboreal and semi-arboreal species, which feed, nest, or escape from predators, at least frequently, by climbing into trees (see **Supplementary Table S2**).

Hibernation Season Duration Data

Reviewing Strategy

Relatively few studies have investigated hibernation duration with body temperature recorders on wild mammals. However, several studies have estimated hibernation season duration from capture-mark-recapture records or direct observations, thus assessing periods of inactivity. Although for some species, the duration of hibernation measured as the period between the first and last torpor bout is probably shorter than the period of inactivity (Young, 1990; Williams et al., 2014; Siutz et al., 2016), these measures should still provide reasonable estimates of the duration of energy savings, thereby allowing species to be compared with each other.

We reviewed the literature to retrieve estimates of the hibernation season duration as the time (in days) spent between immergence and emergence from the hibernaculum or den (with little or no movement outside). The search criteria were based on combining the following terms: "hibernation" OR "hibernation duration" OR "denning" (exclusively for bears) OR "roosting" (exclusively for bats) AND scientific or common names of species. In order to minimize heterogeneity in the scales at which the data were measured (e.g., individual, population), we considered maximum hibernation season duration obtained from same-sex adult groups, either male or female depending on the species (and recorded maximum hibernation season duration from overall population data when more precise data were unavailable).

Inclusion and Exclusion Criteria

We prioritized studies for which the methodology for estimating hibernation season duration was described (capture-mark-recapture, direct observation and body temperature recording). These criteria included 64 species. We also included studies based on road kills as an index of activity/inactivity. This criterion has already been used to evaluate changes in hedgehog abundance (Morris and Morris, 1988; Bright et al., 2015; Wembridge et al., 2016) and was used for 2 species in our data set [The Algerian hedgehog (*Atelerix algirus*) and the Southern white-breasted hedgehog (*Erinaceus concolor*)]. Finally, in cases where the above criteria were not available, we included studies for which a precise hibernation period was mentioned but the methodology could not be assessed. This criterion included 16 species (see **Supplementary Table S2**, labeled species).

We excluded studies for which periods of extreme inactivity were measured only once in a population. This criterion excluded three maximum hibernation season data: 8 months for the little pocket mouse (*Perognathus longimembris*; Kenagy and Bartholomew, 1985); 6 months for the long-tailed pocket mouse (*Chaetodipus formosus*; Kenagy and Bartholomew, 1985); and 11 months for the Eastern chipmunk (*Tamias striatus*; Munro et al., 2008).

The availability or absence of data (longevity and hibernation season duration) in the literature for the 152 species examined is specified in **Supplementary Material** (see **Supplementary Table S1**). In total, our literature search allowed inclusion of 82 hibernating mammals in the analyses including 80 placental mammals, 27 bats, one marsupial (the mountain pygmy possum), and one monotreme [the short-beaked echidna (*Tachyglossus aculeatus*)]. Longevity, body mass and hibernation season duration data for these species are available in **Supplementary Material** (see **Supplementary Table S2**).

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[2] https://genomics.senescence.info/species/search_list.php

[3] https://ecologicaldata.org/wiki/pantheria; Ecological Archives

[4] https://academic.oup.com/mspecies

Phylogenetic Data

We downloaded 100 phylogenetic mammalian trees[5] (see (Upham et al., 2019), focusing on the 82 species in our data set (see **Supplementary Table S2**). These trees were used to construct strict consensus trees for the hibernating species of our study, where the included clades were those present in all the 100 phylogenetic mammalian trees (Paradis, 2011). Because we ran subsequent analyses on different subsets of the global dataset (see section "Phylogenetic Data" below), we constructed separate phylogenetic trees on (1) the full hibernator data set (N = 82 species), (2) the data set excluding bats (N = 55 species), (3) the data set with only deep hibernators (see below) excluding bats (N = 46 species), and (4) the data set excluding bats and hibernators >1.5 kg (N = 44 species) (see **Supplementary Figure S1**).

The rationale for eliminating bats from some analyses was to compare the specific effect of hibernation season duration on longevity between bats which have very distinct characteristics (i.e., flight capacity, highly gregarious behavior during hibernation, Austad and Fischer, 1991) and other hibernators.

The metabolic rate during torpor is not known for all hibernating species studied (Ruf and Geiser, 2015). To test the prediction that the effect of hibernation season duration on longevity should increase with metabolic reduction, we compared the effect of hibernation season duration on the longevity of two groups including (all hibernators excluding bats) or excluding (only deep hibernators without bats) species reducing their energy expenditure during hibernation by less than 90% compared to the euthermic state. These comprise *Ursidae* species and the European badger (*Meles meles*) that reduce their total energy expenditure from 33 to 75% during hibernation compared to the euthermic state (Hellgren, 1998; Watts and Jonkel, 1988; Tøien et al., 2011; Ruf and Geiser, 2015) and small tropical hibernators such as *Cheirogaleidae* and *Tenrecidae* species, which show a 70% reduction (Dausmann et al., 2009; Wein, 2010). Thus, the "deep hibernator" group includes the species capable of reducing their total energy expenditure by about 90% or more during hibernation as compared to the euthermic state and reaching a body temperature during torpor below 10°C (mainly small Holarctic species; Heldmaier et al., 2004).

In addition, the analyses of Turbill et al., 2011 indicated a body mass threshold of 1.5 kg, below which the benefits of hibernation (compared to non-hibernation) for longevity increased. To test the effect of hibernation season duration on longevity between hibernators <1.5 kg and larger ones, we used the data set excluding bats and hibernators >1.5 kg.

Branch lengths for respective consensus trees were calculated with the "compute.brlen" function from the "ape" package based on Grafen's (1989) computations, and were used to compute PGLS models with the "caper" package in R (see section "Phylogenetic Data" below).

Statistics

We tested for a significant relationship between hibernation season duration and species maximum longevity, using phylogenetic generalized least squares (PGLS) models with the "ape 5.0," "apTreeshape 1.5," and "caper 1.0" packages in R v. 3.6.2 (Paradis, 2011; Orme et al., 2013; Paradis and Schliep, 2019; R Core Team, 2019). We thus statistically "controlled" for the influence of the phylogenetic relationships among species on the variables before evaluating relationships. In addition, the relative effect of the phylogenetic tree on the linear model could be estimated as a λ parameter, ranging between 0 (covariation among species measurements is independent of co-ancestry) and 1 (covariance entirely explained by co-ancestry). Testing the models with $\lambda = 0$ allowed comparison to λ-positive models, and thus the extent to which phylogeny influenced analyses of the models examined. In addition to phylogeny, our model evaluated the influences of average body mass (of adults) of the different species, bats and arboreality lifestyle, and the fact that some data were acquired from captive and wild populations (see below).

We ran PGLS models for the four different conditions listed above (**Table 1**). Longevity was the dependent variable in all our models, and hibernation season duration and species average body mass were independent variables. In all models, body mass and longevity were log-transformed to normalize their distributions, and all independent variables were standardized (using *z-scores*), so that their coefficients are directly comparable as estimates of effect sizes (Abdi, 2007). In the original models, we included the interaction *hibernation season duration x body mass* to test for the possibility that the effect of hibernation season duration on longevity was more important for species of small body mass (see **Figure 2** in Turbill et al., 2011), as well as a "captive/wild" factor to account for captivity-related variation in longevity (Tidière et al., 2016). However, these factors were parsimoniously dropped in the final models based on Akaike's Information Criterion (AIC). Among the models within $\Delta\text{AIC} < 2$ ($\Delta\text{AIC}_\text{i} = \text{AIC}_\text{i}\text{-AIC}_\text{min}$), we kept the model with the lowest number of terms (see **Supplementary Table S3**). Nevertheless, body mass was retained throughout our models, because of the dominance that it shows as a primary axis of energetics and life history (Stearns, 1992; Brown et al., 2004). In order to control for higher longevity due to particular lifestyles, we added a "bat (yes/no)" factor in the full model (Wilkinson and South, 2002; Turbill et al., 2011) and an "arboreality (yes/no)" factor in each model (Kamilar et al., 2010; Shattuck and Williams, 2010; Healy et al., 2014).We limited the number of additional predicators in order to maintain sufficient statistical power with respect to the sample size (**Table 2**; Mundry, 2014).

For the final models, the level of covariation in maximum longevity among species was estimated by maximum likelihood (λ_{ML}).

Within bats (individuals from wild populations only), we were not able to estimate the effects of body mass and hibernation season duration on longevity, while controlling statistically for phylogeny. We had too few species of bats (N = 27; Münkemüller et al., 2013) for properly evaluating the phylogenetic signal (the lower CI bound for the phylogenetic signal could not be estimated; see **Supplementary Material**,

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[5] http://vertlife.org/phylosubsets/

TABLE 1 | Summary of models and datasets.

Model	1	2	3	4	5
Model type	**PGLS**	**PGLS**	**PGLS**	**PGLS**	**Linear model**
Hibernators > 1.5 kg	x	x			
Hibernators < 1.5 kg	x	x		x	
Deep hibernators	x	x	x		
Bats	x				x
Sample size	82	55	46	44	27
Arboreal and semi-arboreal species	16	16	11	15	0
Hibernation season duration range (day)	105–296	105–296	105–296	105–296	120–255
Longevity range (year)	3.5–49.5	3.5–49.5	3.5–49.5	3.5–29	6–41
Body mass range (g)	4.6–227500	8–227500	8–7300	8–958	4.6–28.55

The category known as "deep hibernator" includes the species capable of reducing their total energy expenditure by about 90% or more during hibernation as compared to the euthermic state and reaching a body temperature during torpor below 10°C (mainly small Holarctic species; Heldmaier et al., 2004). Crosses indicate group(s) included in each model.

Supplementary Figure S2). Thus, we present simple linear regressions for this group later indicated as model 5 (**Tables 1**, **2**).

RESULTS

The characteristics of the models and data used are summarized in **Table 1**. For each model, hibernation season duration and longevity were similar in range between the different datasets (**Table 1**). Naturally, the range of body mass was much smaller when considering only deep hibernators, small species and bats.

Model 1 (*N* = 82)

Accounting for the effect of phylogeny, variation in longevity was positively associated with hibernation season duration and body mass across all hibernating mammals (**Figures 1**, **2** and **Table 2**). On average, bats had significantly longer lifespans (83%, $\bar{x}$ = 21 years, *SD* = 8.7, *N* = 27) as well as species with an arboreal lifestyle (12.6%, $\bar{x}$ = 12.9 years, *SD* = 9.2, *N* = 16), than other non-flying and non-arboreal mammals ($\bar{x}$ = 11.5 years, *SD* = 9.7, *N* = 39) (**Table 2**).

Model 2 (*N* = 55)

Removing hibernating bats from the analyses showed that hibernation season duration still had a significant positive effect on longevity (**Figures 1**, **2** and **Table 2**). The positive effect of body mass and an arboreal lifestyle on longevity remained, mammals with a higher body mass or an arboreal lifestyle exhibiting significantly longer lifespan (**Figures 1**, **2** and **Table 2**).

Models 3 and 4 (*N* = 46 and *N* = 44, Respectively)

When only deep hibernators (model 3) and small hibernators <1.5 kg (excluding bats) were considered (model 4), we found a positive effect of hibernation season duration on longevity (**Figures 1**, **2** and **Table 2**). The positive effect of arboreality lifestyle on longevity only remained for small hibernators (model 4).

Among the above models, the effect of hibernation season duration was slightly higher for small hibernators <1.5 kg and highly significant (**Figures 1**, **2** and **Table 2**). These models showed a strong influence of phylogeny on the results (**Table 2**). Ignoring the effect of phylogeny by constraining λ to 0 removed the effect of hibernation season duration on longevity (**Table 2**).

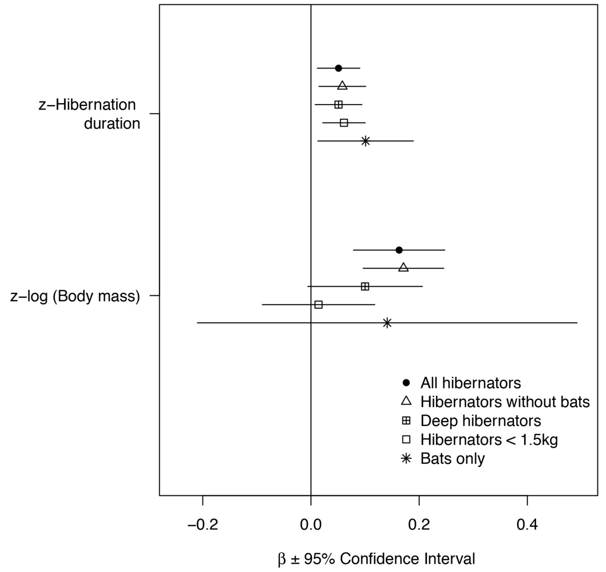

FIGURE 1 | Effects of hibernation season duration and body mass on longevity. Z-standardized model coefficients are presented ±95% Confidence Interval (1.96xSE). Both body mass and longevity were log-transformed before the analyses. The effect sizes are presented for PGLS models for all mammals (*N* = 82), mammals without bats (*N* = 55), deep hibernators without bats (*n* = 46) and small mammals without bats (*N* = 44). For comparison, effect sizes from a simple linear model not accounting for phylogeny are presented for bats only (*N* = 27).

Model 5 (*N* = 27)

Because our sample size for bats alone was too small, we could not perform an analysis controlling for phylogeny. Instead, we ran simple linear models to test for the effects of hibernation season duration and body mass on longevity. Here as well, hibernation season duration, but not body

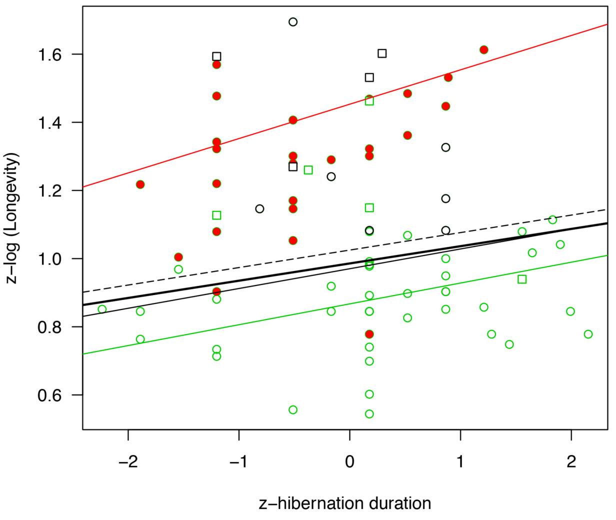

FIGURE 2 | Relationship between hibernation season duration (standardized) and longevity (log-transformed and standardized). The regression lines are presented for PGLS models for all mammals (bold black line, $N = 82$, $p = 0.015$), all hibernators without bats (black line, $N = 55$, $p = 0.011$), deep hibernators without bats (dashed line, $N = 46$, $p = 0.028$) and small hibernators without bats (green line, $N = 44$, $p = 0.005$). For comparison, effect sizes from a simple linear model not accounting for phylogeny are presented for bats only (red line, $N = 27$, $p = 0.036$). Full red circles highlight bat species, the squares highlight species reducing their energy expenditure during hibernation by less than 90% compared to the euthermic state, green item highlight species below 1.5 kg, and black circle highlight all remaining hibernators above 1.5 kg in the data set. Please note that some dots referring to bat species are superimposed because the corresponding species have the same hibernation season durations and longevities.

mass, was positively and significantly related to maximum longevity (**Figures 1**, **2** and **Table 2**). Hibernation season duration had an effect on bat longevity up to two times higher than in previous models excluding bats. This significant effect without accounting for phylogeny was probably due to a lower level of phylogenetic differences among bat species, compared to other models including up to eight different orders.

DISCUSSION

Influence of Hibernation Season Duration on Longevity

Our purpose was to examine the extent to which the activity time budget explains variation in life history traits in mammals. We investigated this question in hibernating species because of their marked seasonal cycle of activity/inactivity, which is associated with very different risks of mortality (Turbill et al., 2011). While controlling for phylogeny, our study highlighted a positive influence of hibernation season duration on longevity in mammalian hibernators. These results were in agreement with an early study that tested the effect of hibernation duration on longevity in captive Turkish hamsters (Lyman et al., 1981). In agreement with Dobson (2007) and Sibly and Brown (2007), the two major axes of life histories of mammalian hibernators are body mass and lifestyle, with lifestyle contributing to the slow-fast continuum. The bat lifestyle (e.g., aerial) had the greatest influence on longevity, with a positive effect on longevity that was three times greater than the arboreality lifestyle. The effect of hibernation season duration, in addition to these lifestyles, appeared to be roughly one-third of the effect of body mass on longevity (see model estimates in **Table 2**).

Our results show that the effects of hibernation season duration on longevity were consistent across a wide range of body sizes, and became stronger with the limitation in body mass to small species (species <1.5 kg), especially for bats (**Figure 1**). Interestingly, hibernation season duration appeared to be more important than body mass in explaining longevity in the latter species. These results support the idea that hibernation (1) is an efficient strategy that limits mortality in periods of energy scarcity for some larger species facing strong energy constraints during part of the year (e.g., *Marmota* species), and (2) may be an especially effective strategy for small mammals that are expected to suffer from both higher predation rate (Cohen et al., 1993; Sinclair et al., 2003) and increased loss of energy expenditure during winter (Ruf and Geiser, 2015). In either case, increased hibernation season duration may increase both annual survival rates (Turbill and Prior, 2016) and overall longevity (this study). Note that in some cases, the lack of relationship between body mass and longevity in our study could also be due to a smaller range of body mass variation than reported in other studies (for instance in bats; Wilkinson and South, 2002).

In our study, the effect of hibernation season duration on the longevity for bats was twice that of small non-flying terrestrial mammals, though this result should be considered with caution since we were not able to control for phylogeny when considering only bats (**Figure 2** and **Table 2**). Bats stand somewhat apart from other mammals, distinguished notably by their ability for sustained flight, an important lifestyle characteristic (Sibly and Brown, 2007). Flying is an energy-intensive activity, considerably more than terrestrial locomotion (Tucker, 1968; Thomas and Suthers, 1972). Thomas and Suthers (1972) estimated that the greater spear-nosed bat (*Phyllostomus hastatus*) increases its resting metabolic rate more than 34 times during flight, while rodents of similar size increase it only by 8-fold during terrestrial locomotion. Thus, in bats the reduction of metabolism during hibernation is particularly important compared to their period of activity (Wilkinson and South, 2002).

The marked effect of hibernation season duration on longevity in bats may also be explained by some extreme physiological adaptations to hibernation having evolved in response to specific ecological and anatomical constraints (Willis, 2017). For instance, several bat species are capable of very long torpor bouts (up to 60 days; reviewed in Ruf and Geiser, 2015), perhaps in response to their limited accumulation of internal or external energy reserves (Willis, 2017). In addition, the little brown bat (*Myotis lucifugus*), for instance, is capable of performing "heterothermic arousals," corresponding to shallow torpor bouts ($T_{skin} > 20°C$), during arousal phases, and thus reduce the cost of euthermia (Jonasson and Willis, 2012; Czenze et al., 2017). This particular adaptation may be

TABLE 2 | Regression results for the best models explaining variation in longevity among hibernators species.

			λ_{ML}				$\lambda = 0$		
			λ_{ML}	**β ± SE**	***t*-value**	***p*-value**	**β ± SE**	***t*-value**	***p*-value**
Phylogenetic correction (PGLS)	Model 1: All hibernators (82 species)	Intercept	λ_{ML} = 0.736 CI_{95} = [NA-0.924]	0.986 ± 0.111	8.847	< 0.001***	0.834 ± 0.032	25.778	< 0.001***
		z-Hibernation duration		0.051 ± 0.020	2.484	0.015*	0.028 ± 0.020	1.343	0.183
		z-log (Body mass)		0.163 ± 0.043	3.800	< 0.001***	0.216 ± 0.026	8.409	< 0.001***
		bat		0.531 ± 0.170	3.131	0.002**	0.656 ± 0.060	10.903	< 0.001***
		arboreality		0.163 ± 0.075	2.175	(0.033*	0.182 ± 0.054	3.387	0.001**
	Model 2: Hibernators without bats (55 species)	Intercept	λ_{ML} = 0.849 CI_{95} = [0.238–0.969]	0.970 ± 0.112	8.682	< 0.001***	0.841 ± 0.031	27.439	< 0.001***
		z-Hibernation duration		0.058 ± 0.022	2.645	0.011*	0.004 ± 0.022	0.200	0.842
		z-log (Body mass)		0.171 ± 0.038	4.442	< 0.001***	0.217 ± 0.024	8.920	< 0.001***
		arboreality		0.191 ± 0.070	2.738	0.008**	0.173 ± 0.051	3.409	0.001**
	Model 3: Deep hibernators without bats (46 species)	Intercept	λ_{ML} = 0.850 CI_{95} = [0.480-0.960]	1.025 ± 0.107	9.611	< 0.001***	0.876 ± 0.026	33.483	< 0.001***
		z-Hibernation duration		0.051 ± 0.022	2.279	0.028*	0.008 ± 0.023	0.342	0.734
		z-log (Body mass)		0.100 ± 0.054	1.833	0.074.	0.173 ± 0.034	5.120	< 0.001***
	Model 4: Small hibernators (<1.5 kg) without bats (44 species)	Intercept	λ_{ML} = 0.740 CI_{95} = [0.293–0.924]	0.867 ± 0.084	10.240	< 0.001***	0.820 ± 0.029	28.543	< 0.001***
		z-Hibernation duration		0.061 ± 0.020	2.994	0.005**	0.024 ± 0.021	1.148	0.258
		z-log (Body mass)		0.014 ± 0.053	0.264	0.793	0.121 ± 0.042	2.864	0.007**
		arboreality		0.204 ± 0.069	2.937	0.005**	0.191 ± 0.048	4.002	< 0.001***
No phylogenetic correction (linear model)	Model 5: Bats only (27 species)	Intercept	Not estimated	1.453 ± 0.168	8.619	< 0.001*		Not estimated	
		z-Hibernation duration		0.101 ± 0.045	2.215	0.036*			
		z-log (Body mass)		0.141 ± 0.179	0.785	0.440			

Z-standardized model estimates (ß) for the effects of hibernation season duration and body mass on species maximum longevity. The phylogenetic effect is estimated by λ_{ML}. Both body mass and longevity were log-transformed before the analyses. For comparison, we have provided estimates for λ constrained to zero (no effect of phylogeny). The model for bats was a simple linear model not controlling for phylogeny, due to limited sample size in this group. In the model with all hibernators, the NA value in the confidence interval for λ_{ML} indicates that the caper package could not calculate the full confidence interval. An NA value is considered as 0. Significant results are represented as $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

present in other bat species as well. Finally, bats are highly gregarious during hibernation and can cluster in colonies of up to thousands of individuals (Clawson et al., 1980). Huddling could enable them to reduce energy costs and water loss during hibernation, making hibernation a particularly profitable strategy (Boyles et al., 2008; Gilbert et al., 2010; Boratyński et al., 2012, 2015).

Comparisons between models either including (model 2) or excluding (model 3) species with the lowest metabolic reductions during hibernation did not reveal significant differences. These results suggest that the effect of hibernation season duration on longevity remains consistent whatever the rate of metabolic reduction reached during hibernation compared to the active state (between 70 and 90%). This finding should pave the way for future studies to specifically test this effect of metabolic reduction during hibernation.

Interestingly, in all PGLS models, removing the effect of phylogeny by constraining λ to 0 also removed the effect of hibernation season duration on longevity. This suggests that the effect of hibernation season duration on longevity is masked by the phylogenetic pattern. Thus, hibernation season duration might be a stronger explanation of variation within species or between closely related species, as shown in Turkish hamsters (Lyman et al., 1981). For instance, studies comparing populations of golden-mantled ground squirrels (*Callospermophilus lateralis*) and Columbian ground squirrels (*Urocitellus columbianus*) living along an altitudinal gradient show that populations with longer hibernation season duration generally have higher annual survival and longevity (Bronson, 1979; Murie and Harris, 1982; Dobson and Murie, 1987).

Although our results highlight an association between hibernation season duration and longevity, they do not provide a causal mechanism through which such an association might arise. Periods of prolonged inactivity are likely to increase longevity through the integration of multiple factors affecting both intrinsic and extrinsic mortality, as discussed below.

Factors Affecting Extrinsic and Intrinsic Mortality and the Evolution of Hibernation

Energetic stress, when energy demand is greater than energy availability in the environment, has a proximate role in the regulation of hibernation pattern (Vuarin and Henry, 2014). However, few studies have focused on the causal link between energetic stress, and the timing of hibernation immergence and emergence (e.g., Humphries et al., 2002). Thus, the hypothesis that hibernation occurs primarily as a response to an energetic stress has not been completely studied (focusing on torpor bouts frequency, depth and duration). To the best of our knowledge, the only study having measured both energy availability in the environment and individual energy expenditure before immergence in hibernation shows in eastern chipmunks (*Tamias striatus*), that immergence occurs while food is still plentiful and climatic conditions are still favorable for maximizing energy storage (Humphries et al., 2002). Other observations also suggest that immergence into hibernation while food is still available seems common, at least in sciurids (Humphries et al., 2003) and for the little pocket mouse (Barnes and Carey, 2004). Thus, food availability and ambient temperature alone may not be sufficient to explain the phenology of immergence.

Other evidence suggests that hibernation is not initiated solely in response to deficiencies in energy, water, or poor food quality. Many observations suggest that early immergence (before energetically stressful periods start) occurs when the benefits of reproduction are low. For instance, in years of low beech seed abundance, the edible dormouse (*Glis glis*) skips reproduction, quickly accumulates fat reserves, and is able to hibernate for up to 11 months (Hoelzl et al., 2015). This occurs even if food in the environment is sufficient to allow the edible dormouse to remain active but not to reproduce. Similarly, Eastern chipmunks skip reproduction and cease foraging for almost a full year when food availability is particularly low (Munro et al., 2008). It seems that at that time, chipmunks rely on large amounts of food hoarded during the preceding year; but there is no evidence of torpor expression. This kind of behavior is also observed in several hibernating ground squirrel species. Females that fail to reproduce may immerge up to several weeks before the others (Michener, 1978; Choromanski-Norris et al., 1986; Bintz, 1988; Neuhaus, 2000). An experiment in semi-natural conditions shows that female European ground squirrels (*Spermophilus citellus*) that were separated from males (and thus did not breed) entered into hibernation 4–6 weeks before females that were not separated (Millesi et al., 2008). These results support the view that hibernation phenology is influenced by a trade-off between reproduction and survival, where hibernation seems to provide benefits other than surviving periods of energetic stress. This trade-off may also explain differences (up to 1 month) in the timing of immergence and emergence between gender and age observed in rodents (Snyder et al., 1961; Holekamp and Nunes, 1989; Kawamichi, 1989; Sheriff et al., 2011; Kart Gür and Gür, 2015; Siutz et al., 2016) and bats (Stebbings, 1970; Thomson, 1982; Decher and Choate, 1995; Norquay and Willis, 2014).

So far, most studies have focused mainly on temperate hibernating species. However heterothermy that occurs during daily torpor and hibernation, is taxonomically and geographically widespread (Ruf and Geiser, 2015). A surprisingly large proportion of mammals, including a monotreme, several marsupials, and placental species regularly enter daily torpor and seasonal hibernation in the southern hemisphere (Grigg and Beard, 2000). For some species in these regions, the use of torpor may not be related to low environmental temperatures or limited food availability (Nowack et al., 2020). For example, the short-beaked echidna hibernates (Grigg et al., 1989) while ants and termites, which constitute the main part of its diet, remain available throughout the year (Grigg and Beard, 2000).

Short-beaked echidnas are heavily armored, perhaps rendering avoidance of predation an unlikely adaptive benefit for hibernation. These observations suggest that some species may use hibernation because of the energy advantages provided by lack of activity, even though it is not an energy necessity for survival during a period of energetic stress (Grigg and Beard, 2000). Such case studies broaden the scope of possibilities for understanding the evolution of hibernation (Grigg and Beard, 2000; Ruf et al., 2012), and open up exciting perspectives for future research.

CONCLUSION

Hibernation is considered an adaptation to seasonal, hence predictable decreases in food resources and ambient temperatures. However, hibernation is also observed in mild climates and when ambient conditions are still favorable for activity (Nowack et al., 2020). If remarkable physiological aspects of hibernation have been widely studied, fewer studies have focused on its ecological and evolutionary significance. Our study provides evidence that there may be a relationship between activity time budgets, hence the time dimension of allocation trade-off, and life history traits.

Our phylogenetic analyses show that variations in hibernation season duration can partially explain variations in longevity in hibernators. The models show a strong influence of phylogeny on this relationship and highlight the need for in-depth studies at an inter- and intra-population scales. For example, future studies may attempt to consider activity time budgets in the context of the pace of life syndrome by examining variations in hibernation with other physiological and behavioral traits. Our results, combined with information available in the literature, suggest that, in addition to its survival benefits during a period of energetic stress, hibernation season duration may have evolved to reduce the effects of other sources of extrinsic and intrinsic mortality.

AUTHOR CONTRIBUTIONS

TC conceived and drafted the manuscript and collected the data. SG and CH contributed to development of the concept and writing of the manuscript. TC, VV, and FD carried out the data analyses. VV, MT, PB, FD, CH, and SG substantially contributed to the conception. FD contributed to the English composition with many revisions of the manuscript. All authors contributed to the revisions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00706/full#supplementary-material

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PERSPECTIVE
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Flexibility Is Costly: Hidden Physiological Damage From Seasonal Phenotypic Transitions in Heterothermic Species

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Heterothermy allows organisms to cope with fluctuating environmental conditions. The use of regulated hypometabolism allows seasonal heterothermic species to cope with annual resource shortages and thus to maximize survival during the unfavorable season. This comes with deep physiological remodeling at each seasonal transition to allow the organism to adjust to the changing environment. In the wild, this adaptation is highly beneficial and largely overcomes potential costs. However, researchers recently proposed that it might also generate both ecological and physiological costs for the organism. Here, we propose new perspectives to be considered when analyzing adaptation to seasonality, in particular considering these costs. We propose a list of putative costs, including DNA damage, inflammatory response to fat load, brain and cognitive defects, digestive malfunction and immunodeficiency, that should receive more attention in future research on physiological seasonality. These costs may only be marginal at each transition event but accumulate over time and therefore emerge with age. In this context, studies in captivity, where we have access to aging individuals with limited extrinsic mortality (e.g., predation), could be highly valuable to experimentally assess the costs of physiological flexibility. Finally, we offer new perspectives, which should be included in demographic models, on how the adaptive value of physiological flexibility could be altered in the future in the context of global warming.

Keywords: flexibility, seasonal transitions, heterothermy, hypometabolism, physiological costs, aging

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INTRODUCTION

In seasonal environments, resource availability fluctuates over the year, mainly between a phase of resource abundance and a phase of resource shortage. Heterothermy is an adaptation to these seasonal changes, which are mainly periodic and therefore predictable, in environmental conditions. Heterothermy is defined as "the pattern of temperature regulation" in an endothermic species (i.e., mammal or bird) "in which the variation in core temperature, either nychthemerally or seasonally, exceeds that which defines homeothermy" [IUPS Thermal Commission (The Commission for Thermal Physiology of the International Union of Physiological Sciences), 2003]. To cope with seasonal fluctuations, heterothermic species show great variations in their metabolic activity. They alternate between an active/reproductive state with a high metabolic rate during the favorable phase and an inactive/resilient state with a low metabolic rate and reduced responsiveness to stimuli [i.e., state of torpor; IUPS Thermal Commission (The Commission for Thermal Physiology of the International Union of Physiological Sciences), 2003] during the unfavorable phase (Gwinner, 1986; Ruf et al., 2012; Ruf and Geiser, 2015). In this article, we chose to focus exclusively on seasonal heterotherms (hereafter abbreviated as "SH") 'that employ hypometabolism [. . .] on a seasonal basis' (Lyman et al., 1982; Ruf and Geiser, 2015), as opposed to opportunistic heterotherms that use hypometabolism as an emergency life-history stage (Wingfield, 2003; Nowack and Dausmann, 2015). There is a difference between obligatory seasonal remodeling (predictable; synchronized with the photoperiod) and unpredictable environment-dependent adjustment (corresponding to the plasticity of opportunistic expression of heterothermy). SH use seasonal changes in the photoperiod as an environmental cue to synchronize their physiology to the environment and to adjust their metabolic rate to undergo either hibernation (i.e., with torpor episodes lasting weeks) or daily torpor during unfavorable phases (Geiser, 2017). Photoperiod-induced changes in metabolic activity (and reproduction) are so deeply imprinted in these species' genomes that we still observe them in captivity where resources are constantly abundant, even after many generations (e.g., Perret et al., 1998). This predominant role of the photoperiod does not preclude the roles of other environmental cues, e.g., food availability, in the expression patterns of heterothermy (Vuarin et al., 2015). SH show specific changes in several physiological and behavioral functions between seasons. For instance, torpor use is linked with the alteration of cell membrane function, neural and cardiovascular functions, skeletal muscle function, immune function, anorexia and nonshivering thermogenesis, or lipidic metabolism and fatty acid saturation (Carey et al., 2003; Klug and Brigham, 2015).

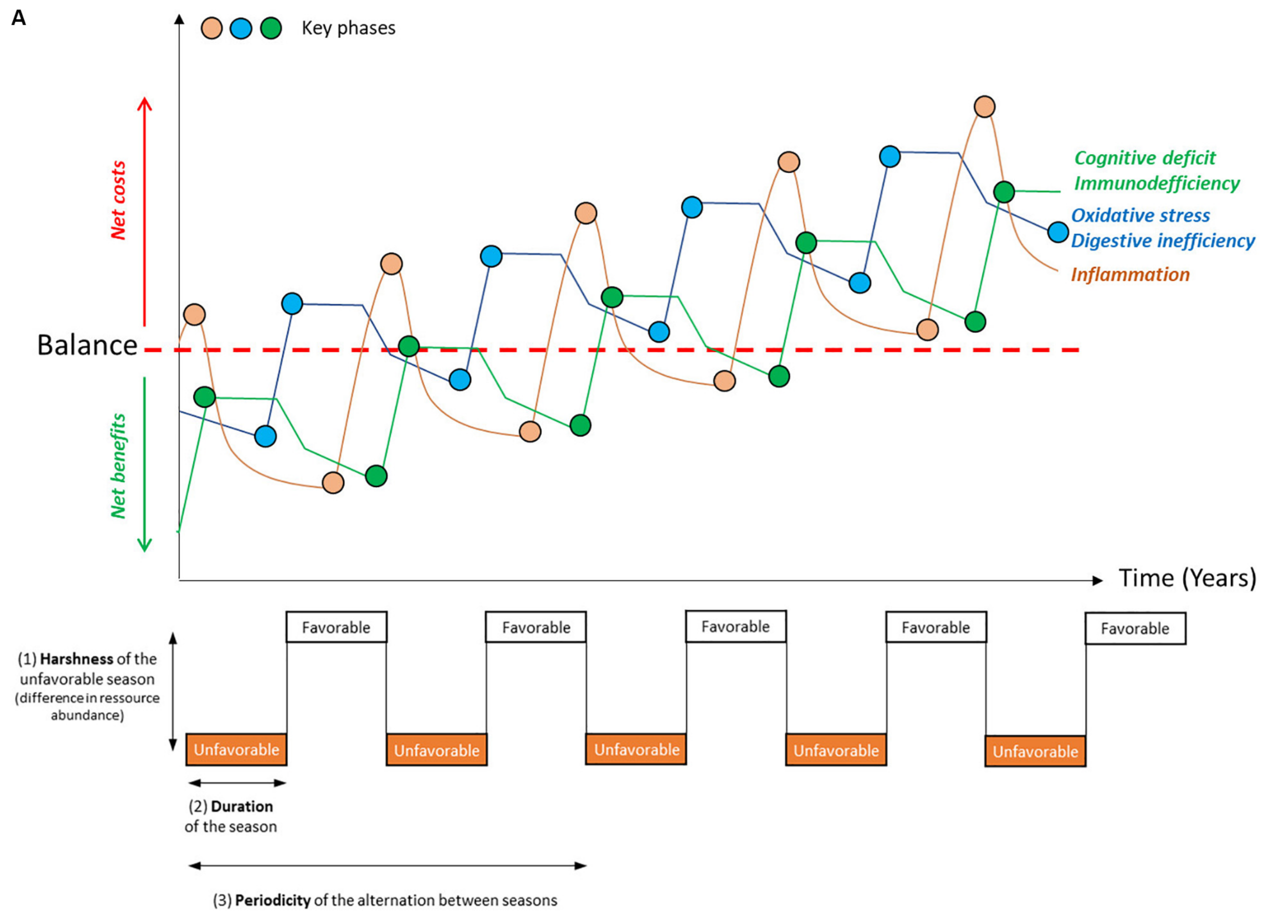
Heterothermy comes with both ecological and physiological benefits (Nowack et al., 2017). Overall, these benefits have to largely exceed the potential costs in fluctuating environments (Lovegrove, 2012; Lovegrove et al., 2014). However, these costs have been largely neglected by researchers. Yet it is important to consider these costs for several reasons. First, understanding how physiological transitions affect both benefits and costs in survival and reproduction and when they occur throughout life is crucial to assess the effects of environmental fluctuations (in harshness, duration and periodicity, see **Figure 1A**) on individual fitness. Second, understanding the constraints linked to heterothermic life cycles (i.e., the covariance between life-history traits resulting from the interactions between environmental conditions and physiological transitions) will allow us to better integrate heterothermic strategies within life-history theory.

In this paper, we propose new perspectives on the marginal costs of seasonal flexibility. We do not intend to provide an extensive review of the literature on the regulation of seasonal heterothermy but rather to shed light on putative physiological costs that have been overlooked so far and should be considered in future research. We propose that these costs should be better supported by experimental evidence to be further included in studies modeling the interactions between heterothermy and environmental changes (Boyles et al., 2020).

THE OBVIOUS ECOLOGICAL BENEFITS OF SEASONAL HETEROTHERMY. . . AND ITS COSTS

Heterothermy brings ecological benefits. First, matching metabolic activity with resource availability maximizes survival, as the organism is less constrained by energy and water shortages. The match of metabolic activity to resource fluctuations in SH also enhances reproductive success. Second, heterothermy promotes metabolic flexibility, i.e., the ability to adjust the origin of substrates used for oxidative metabolism, switching from carbohydrate to lipid usage. Metabolic flexibility is thought to enhance the control of energy balance and has even been proposed to favor longevity (Goodpaster and Sparks, 2017; Smith et al., 2018). Regulated hypometabolism (as opposed to unregulated, pathological hypothermia; Barros et al., 2001) has been shown to increase survival and to lower the senescence rate during the unfavorable season (e.g., Liow et al., 2009; Turbill et al., 2011, 2012). It also often correlates with reproductive inactivity (e.g., Canale et al., 2012), which, according to the disposable soma theory of aging (Kirkwood and Holliday, 1979), could also promote repair mechanisms and slow down the senescence process, as energy is not invested in reproduction (Ricklefs and Wikelski, 2002).

However, seasonal heterothermy may also come with ecological costs. In the wild, these costs are compensated by the benefits of heterothermy and are therefore hidden (not observable). However, several recent studies revealed such costs. For example, some species avoid using torpor when environmental conditions are good enough (e.g., Landry-Cuerrier et al., 2008; Levy et al., 2011). This suggests that the benefits of using torpor only hold in unfavorable conditions and that expression of torpor when not necessary might come with costs. Organisms using heterothermy are also shown to perform poorly in terms of resource acquisition because of a phenological trophic mismatch. For example, mice using torpor emerge later than nontorpid mice and "miss" the period of good resource availability (Levy et al., 2012). When they emerge, competition for food is high, and the best resources are no longer available. Therefore, mice using torpor show less energy intake and compensate by using longer torpor periods, which contribute to maintaining the mismatch. Another potential ecological cost of heterothermy is increased predation risk during torpor and at the beginning of the active period due to decreased locomotor ability and reduced perception of the environment (Humphries et al., 2003; Carr and Lima, 2013), although torpor use also reduces exposure to predators (e.g., Turbill and Stojanovski, 2018). Torpor also leads to dehydration, which implies compromised circulation and modification of the ionic balance (Humphries et al., 2003). The decrease in locomotor abilities associated with torpor use also leads to an increase in hoard pilferage risk for food-storing SH (Humphries et al., 2003).



B

When and how assess for the hypothesized physiological costs

Oxidative stress
Digestive inefficiency

Physiological threats

- Unbalanced oxidative stress and genomic instability
- Altered digestive flexibility leading to malnutrition

Key phases

- Low oxidative stress and repair mechanisms / low microbiota and digestive capacity during unfavorable season
- Increase of oxidative stress and microbiota diversity at arousal from unfavorable period

Examples of methods to assay the costs*

- Assays of makers of oxidative status (8-OHdG, hydroperoxides, biproducts of lipid/protein peroxidation) and of antioxidant activity (Thiols, plasma anti-oxidant barrier, Gluthatione peroxidase) from blood using ELISA or colorimetric assays.
- Measure of relative telomere length (RTL) from blood/skin biopsy/buccal smear using qPCR.
- Measure of gut microbiota diversity/quantity from stools using genomics.

Inflammation

Physiological threats

- Systemic inflammatory signals leading to metabolic dysfunction (obesity and diabetes)

Key phases

- High levels of fat mass in the fattening period preceding the unfavorable condition
- Low levels of fat mass during the active/reproductive season

Example of methods to assay the costs*

- Measures of fat mass
- Measures of blood glucose and insulin levels.
- Assay of circulating pro-inflammatory (IL1, IFNg, TNFa, etc) or anti-inflammatory cytokines (IL-4, IL-6, IL-10, etc), of adipokines, etc

Cognitive deficit
Immunodefficiency

Physiological threats

- Alteration of brain function leading to altered cognitive/behavioral capacities
- Altered immune capacity leading to increased pathogen load

Key phases

- Low cognitive and immune function at the very end of unfavorable season
- Increase of cognitive and immune functions at the beginning of the active season

Examples of methods to assay the costs*

- Description of locomotor trajectories to assess memory
- Assays of circulating markers of brain dysfunction (e.g. axonal protein neurofilament light NFL) and neurodegeneration (e.g. urine exosomes)
- Analysis of blood smears to assess for immune cells distribution.
- Assays of aspects of innate immune function (e.g. natural antibodies, complement, PIT54/lysozyme acute phase protein

*Proposed methods are supposed to be applicable in field conditions. Other methods are available in experimental set-ups

FIGURE 1 | (**A**-Top panel) schematic representation of the hypothetical changes over time in the cumulative damage induced by different physiological costs of heterothermy and the corresponding key phases when these costs are likely to occur. These changes account for phases of bursts and phases of uncomplete restoration of the considered damages. The red dotted line represents the threshold at which the balance between the benefits and the costs for each considered physiological feature is null. Each cost emerges early in life but is only partially compensated for, therefore leading to the accumulation of damage with age. (**A**-Bottom panel) parameters, i.e., harshness (1), duration (2), and periodicity (3), involved in the seasonal transitions undergone by heterothermic species. **(B)** Examples of physiological costs of heterothermy, the key time phases at which they may occur, and proposed methods to assay these costs.

PHYSIOLOGICAL COSTS OF HETEROTHERMY EMERGE EARLY IN LIFE AND ACCUMULATE WITH AGE

There is an apparent paradox between the fact that seasonal heterothermy is an adaptation to predictable environmental changes and the recent hypothesis that the associated circannual physiological changes are detrimental to the organism in terms of increased mortality and early emergence of aging phenotypes (Landes et al., 2017). Indeed, the seasonal transitions between inactive and active metabolic states, as manifestations of phenotypic plasticity, come with a major remodeling of organismal physiology. For example, the changes in metabolic rate and body temperature are due to finely tuned modifications, such as changes in the expression of enzymes involved in anabolic and catabolic metabolism, erythropoiesis, fuel sources, regulation of protein transcription, membrane composition and thermogenesis (Ramenofsky and Wingfield, 2007; Klug and Brigham, 2015; Ruf and Geiser, 2015). This remodeling comes with generic costs of phenotypic plasticity referred to as maintenance, production, information acquisition, developmental instability and genetic costs (reviewed by Auld et al., 2010). Such costs may appear early in life, at key phases where they could be measured (**Figure 1**) and may affect mortality (**Box 1** and **Figure 2A**; Landes et al., 2017).

Experimental Evidence of the Costs of Seasonal Physiological Remodeling

Increased frequency of seasonal transitions has been linked to increased mortality in captive gray mouse lemurs (*Microcebus murinus*; Landes et al., 2017). In this study, individuals were exposed to different numbers of seasonal transitions per year. Those experiencing more frequent seasonal transitions than usual showed an increase in mortality that was already visible at a very young age, therefore potentially contributing to faster aging (see **Box 1**). Functionally, increasing the frequency of seasonal transitions is known to lead to disorders, such as the emergence of altered circadian rhythmicity similar to the ones exhibited by aged individuals under a natural seasonal rhythm (Cayetanot et al., 2005). Interestingly, the animals that were put on accelerated seasonal rhythms (up to five seasons per year instead of two) were all properly "seasonally" entrained in terms of many of their physiological features (e.g., body mass, body temperature, reproduction, brain function) and did not show any sign of discomfort or illness. However, further increases in the frequency of seasonal transitions (i.e., more than five transition events per year) clearly showed that animals were not responsive to the change in photoperiod and escaped to the imposed seasonal rhythms as a manifestation of photorefractoriness (unpublished data; Martine Perret, personal communication). This demonstrates that the remodeling that occurs at each transition requires time and that there is a limit to the number of times that the organism can adjust. In addition, an increased frequency of seasonal transitions may increase the associated costs, as animals showed a more rapid decline with age in melatonin production and in their suprachiasmatic response to light (Aujard et al., 2001).

Repair Mechanisms Are Altered During Aging

Extreme physiological performance ("profound physiological remodeling" in Regan et al., 2019) jointly evolved with specific physiological compensatory mechanisms that protect from and/or restore inevitable somatic damage. For instance, if an extreme metabolic performance generates above-normal oxidative stress, it usually comes along with the overexpression of enzymes that protect against/or repair DNA and cell membrane damage (**Figure 1A**). Aging results from the accumulation of damage to the organism over the lifetime, for which

BOX 1 | Potential demographic effects of seasonal transitions on aging.

We discuss here several possible ways in which frequencies and magnitudes of seasonal physiological transitions may shape mortality over age.

Figure 2A depicts the case where doubling the frequency of transition each year has a proportional hazard effect (PH) on mortality. This was observed in female gray mouse lemurs (*Microcebus murinus*) by Landes et al. (2017). In this case, the mortality of individuals subjected to accelerated seasonal transitions was multiplied by a factor independent of age (i.e., a relative risk). As a consequence, mortality rose faster in accelerated individuals (the slope $h'(t)$ of mortality at a given age was increased). This supports accelerated aging in line with empirical observation that accelerated individuals exhibit early appearance of phenotypes and pathologies associated with aging (Aujard et al., 2001; Cayetanot et al., 2005). However, in this case, the rate of aging $h'(t)/h(t)$ (i.e., how much mortality increased with age with respect to the level of mortality at this age) was constant.

An alternative effect of seasonal transitions could have been an accelerated failure time effect (AFT, **Figure 2B**). In this case, the frequency of transition changed how the time unit affected mortality and changed the parameter b of the Gompertz function. For instance, 1 year of time of reference individuals was equivalent to 2 years for accelerated individuals in **Figure 2B** and, consequently, accelerated individuals senesced twice as fast as reference individuals. Accelerated individuals clearly aged faster here since both $h'(t)$ and $h'(t)/h(t)$ were larger in accelerated individuals. This model would have been a perfect fit for the expectation that increasing transition frequency accelerates biological aging. This was, however, not supported by the data in Landes et al. (2017), even when testing for a combination of both PH and AFT effects (as in **Figure 2C**) following the statistical formulations in Landes et al. (2017), Supplementary Material, Appendix 2). Although such an AFT effect was not evidenced in Landes et al. (2017), this hypothesis still seems probable and should be further explored in future research.

Let us now focus on changes in the magnitude of mortality fluctuations with age. **Figure 2D** depicts the case where the magnitude of mortality fluctuations declines with age in accelerated individuals. This was observed in male gray mouse lemurs (alternative PH-(negative)-AFT model in Landes et al., 2017, supplementary table 2). Here, young adult accelerated males had, as did females, a proportional increase in mortality with age, but mortality increases fast in the young ages and then more and more slowly while individuals age ($\beta < 0$). It seems therefore that because males' seasonal transitions had a lower amplitude with age (illustrated by reduced body mass fluctuations), the cost in terms of mortality also decreased with age. This is interesting because, if proven true, this would be a case where deterioration of the ability to perform seasonal transitions with age leads to a decreasing aging rate. To better emphasize the originality of these results, we provided in **Figures 2E,F** two alternative models that should fit the data if senescence of physiological transitions leads to an increase in the magnitude of mortality fluctuations without (**Figure 2E**) or with (**Figure 2F**) a positive AFT effect on the aging rate (although the models did not fit the data in Landes et al., 2017).

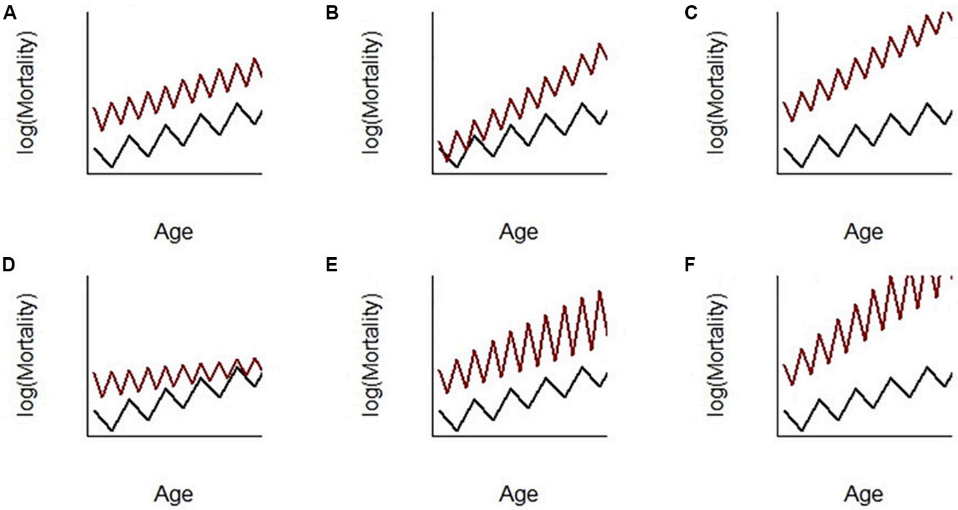


FIGURE 2 | Scenarios for the potential effect of seasonal transitions (in frequency and magnitude) on mortality patterns with age. Age-specific mortality *h(t)* is modeled by a Gompertz-shaped function such that the mortality hazard at age *t* is $h(t) = (ae^{sr.\alpha})(e^{bte^{sr.\beta}})(e^{s.(\gamma t+\sigma)})$, where (i) the first term is the proportional hazard (PH) component of the Gompertz parameter *a*, with *sr* (for seasonal rhythm) being the covariate describing the frequency of the alternation between seasons and α being its PH effect; (ii) the second term is the accelerated failure time (AFT) component of the Gompertz parameter *b*, with β being the AFT effect of variable *sr*; (iii) the third term is the effect of the Boolean variable season *s* on mortality, with a magnitude σ and a potential increase or decrease of the magnitude of fluctuations with age *t* according to the sign of γ. **(A)** PH effect of *sr* on mortality; **(B)** positive AFT effect of *sr* on mortality; **(C)** combined PH and positive AFT effect; **(D)** PH and negative AFT effects with a decreased magnitude of fluctuations with age; **(E)** PH effect with an increased magnitude of fluctuations with age; **(F)** PH and positive AFT effects with an increased magnitude of fluctuations with age.

repair mechanisms no longer fully and efficiently compensate. Indeed, damage accumulates during each season, but repair processes (e.g., antioxidant enzymes in the case of oxidative stress) compensate, at least partially, for this damage to restore proper function at the adult stage (**Figure 1A**). In this case, the net benefits of heterothermy fully balance the costs, which are, therefore, not necessarily observable. Because the ability to perform this damage compensation decreases with age, for example, at the mitochondrial (Babbar et al., 2020) or genomic (Petr et al., 2020) levels, and because of the progressive accumulation of this damage, seasonal organisms may not suffer from the cost of physiological transitions before advanced age. Therefore, as compensation is not fully complete at each seasonal transition (**Figure 1A**), damage emerges early and further accumulates over age to reach a limit where the net costs overcome the benefits (**Figure 1A**). This principle of balance between damage and protective/repair responses applies to the different hypothetical physiological cost mechanisms listed hereafter. However, the cost of transitions would not be easily observed in the wild, where old ages are not often reached. Moreover, the ecological and physiological benefits of torpor expression hide these potential costs in the wild, a feature that does not hold in captivity where environmental constraints are mild. However, the study of individuals in captivity, where extrinsic mortality is negligible and more individuals reach advanced ages, promotes the observation of such costs. Captivity also allows control of photoperiodic entrainment and therefore facilitates access to animals reaching key phases when costs may arise and thus facilitates their assessment (**Figure 1**). Moreover, the links between seasonal transitions and aging are not trivial and need further investigation (see **Box 1**).

Heterothermy Affects the Aging Process

In SH, such as the gray mouse lemur, the mortality pattern over age is driven by seasonality. The link between seasonal transitions and aging is interesting, as aging can be interpreted as the manifestation of damage accumulated at multiple levels. Indeed, seasonal physiological transitions must affect one or several biological functions, therefore contributing to the senescence process, which becomes apparent with the assessment of biological markers of aging (López-Otín et al., 2013). Some of these markers directly show the cause of the accumulating cellular damage, such as genomic instability, telomere attrition or epigenetic alterations. Other markers are the response of the organism to accumulating damage that becomes deleterious after a certain age, such as the loss of proteostasis, deregulation of nutrient sensing, mitochondrial dysfunction or cellular senescence.

Experimentally, regulated hypometabolism can be triggered in different ways, including food shortage or caloric restriction, thus mimicking phases of food scarcity in wild conditions (Vuarin and Henry, 2014). In this context, caloric restriction experiments have also evidenced hidden costs of regulated hypometabolism. These experiments have been extensively studied to manipulate intrinsic functioning and the pace of senescence in a large variety of species, including primates (Mattison et al., 2017; Pifferi et al., 2018). These studies showed that organisms under caloric restriction exhibit a decrease in metabolic rate and energy expenditure (DeLany et al., 1999; Redman et al., 2018), an enhanced lifespan and a delayed emergence of biomarkers of aging and age-related phenotypes (Mattison et al., 2017; Pifferi et al., 2018). However, these studies did not yet provide evidence on the extent to which metabolic depression following caloric restriction mimics a reduction in the pace of life and/or influences the speed at which mortality increases with age. In contrast, other studies suggest that increased mitochondrial uncoupling may also decrease oxidative stress and increase longevity (e.g., Caldeira da Silva et al., 2008). However, different studies support the hypothesis of an energy trade-off between somatic maintenance and reproduction during caloric restriction (e.g., Sitzmann et al., 2008, 2010; Pifferi et al., 2018), which may also hold during seasonal hypometabolic episodes. This is further supported by empirical data showing a negative correlation between the use of regulated hypometabolism and reproductive success in hibernating eastern chipmunks (Dammhahn et al., 2017).

Thus, adaptation to seasonal environments comes at a cost, which might translate into increased mortality risk. However, the underlying mechanisms linking the costs of seasonality and survival remain misunderstood. Here, we provide a list of putative physiological costs that should be further considered in experimental work to determine whether these costs are real and could contribute, to some extent, to minimizing the benefits of heterothermy and further accelerating the rate of aging.

PUTATIVE PHYSIOLOGICAL COSTS OF HETEROTHERMY

We expect physiological costs of seasonal heterothermy to emerge from imperfect biological regulations, imperfect seasonal regulation of competing functions and from suboptimal phenotypic matching with environmental changes. This recurrent phenotypic remodeling contributes to the accumulation of damage and thus to the aging process in SH (see **Figure 1A**). Although experimental evidence on each of these costs is very weak, the associated deep physiological remodeling has to be costly (Auld et al., 2010). Here, we will discuss five types of potential physiological costs of heterothermy that seem especially potent and relevant according to our knowledge on seasonality in SH. Indeed, in addition to their relevance from ecological perspectives, these five physiological costs are hot topics in other disciplines, including biomedical research (e.g., anti-inflammatory and antioxidant mechanisms), which would be interesting to take into account when studying heterothermy. For each cost, **Figure 1** proposes an example of the potential evolution with time of the associated damages (**Figure 1A**), including phases of bursts and of incomplete restoration of damages and the key time phases at which these costs may occur (**Figures 1A,B**). We also provide some methodological examples that could be used to assay these costs in captivity and that are applicable to the field (**Figure 1B**).

Oxidative Stress and Genomic Instability

As mentioned earlier, biological aging is mechanistically linked with metabolism since cellular functioning generates free radicals that cause cellular damage (Selman et al., 2012). The excess of free radicals compared to the cellular antioxidant response leads to oxidative stress. Under such circumstances, organisms experience a loss of functionality and a loss of cellular control, linked, for example, with cancer emergence when cells develop resistance capacities to high levels of oxidative stress (Sosa et al., 2013). Thus, the effect of oxidative stress on senescence depends on the balance between damage and molecular/cellular repair efficiency. As a consequence, the observed oxidative stress can be either directly the cause of DNA damage accumulation with age (i.e., genomic instability, telomere attrition, or epigenetic alterations), the responses to the damages becoming deleterious with age through impaired cellular processes (i.e., loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, or cellular senescence), or phenotypic results of the two previous phenomena leading to functional decline (i.e., stem cell exhaustion and altered intercellular communications; López-Otín et al., 2013). In SH, regulated hypometabolism is linked with low oxidative stress, such as in Arctic ground squirrels (Orr et al., 2009). In addition, variations in relative telomere length (a marker of aging) in SH model species show an elongation of telomeres during the hypometabolic state (Turbill et al., 2013). Telomere attrition is a marker of biological aging and apparently compromises DNA integrity; oxidative stress is thus low during regulated hypometabolism. However, arousal from a hypometabolic state may generate a brief but massive peak in oxidative damage (**Figure 1**). In mouse lemurs, when metabolic activity increases, DNA oxidative damage increases and relative telomere length decreases (Terrien et al., 2017). Telomere shortening during this seasonal transition may be an adaptive response. Indeed, this attrition would contribute to the amplification of the signaling of metabolic debt and therefore to the prioritization of somatic maintenance processes (Casagrande and Hau, 2019). In this respect, telomerase, whose activity varies across species, cell types and life stages (Gomes et al., 2010), represents the most widespread repair mechanism in normal cells. Interestingly, while telomerase activity in somatic tissues has been lost in some species, including humans, there is experimental evidence of maintained telomere repair capacity in normal somatic cells in SH (Wang et al., 2011; Gorbunova et al., 2014; Trochet et al., 2015). One could therefore hypothesize that maintenance of telomerase activity in somatic tissues is one feature of SH and that this repair mechanism might concur with extended longevity compared to that of homeotherm species (Turbill et al., 2011). However, although major focus has been placed on the potential of telomerase reactivation in anti-aging strategies, there exists little evidence that telomerase activity is impaired with age and that this translates into altered repair capacity (Anchelin et al., 2011).

Excess Fat and the Associated Inflammatory Response

Another potential physiological cost of heterothermy is the risk associated with massive variations in body condition and, more particularly, fat reserves (**Figure 1**). For instance, SH often anticipate the harsh season by experiencing massive fattening (+50 to +100%) to build energy reserves on which they will rely for weeks or months. What remains exceptional, and not fully understood, is their faculty to store massive amounts of fat with no adverse effects. Briefly, excess fat, inducing metabolic imbalance, usually induces chronic low-grade systemic inflammation (Lumeng and Saltiel, 2011), leading to increasing circulating levels of proinflammatory and neurotoxic mediators. These signals migrate to the brain to trigger neuroinflammation, preceding the onset of obesity and insulin resistance (Thaler et al., 2012). In SH, a paradox is emphasized, as insulin sensitivity seems to be preserved during the obesogenic phase, which could be mediated by altered adipose PTEN/AKT signaling (Rigano et al., 2017). Recent work on the mouse lemur has shown that the relative expression of phospho-IRS-1 was enhanced in muscle during torpor but decreased in white adipose tissue, thus suggesting an inhibition of insulin/IGF-1 signaling during torpor in these tissues (Tessier et al., 2015). In parallel, animals seem to be protected from an inflammatory response during massive fattening (Terrien et al., 2017). Further work is needed to determine to what extent the mechanisms that prevent insulin resistance and inflammation during fattening are altered with age.

Cognitive and Brain Dysfunction

During regulated hypometabolism, locomotor and sensory capacities are decreased temporarily (**Figure 1**), which is linked with reversible cerebral disorders (tau phosphorylation, loss of neuron functionality) and sleep debt (impaired memory consolidation; Royo et al., 2019). Among the exceptional features of SH, the homeostasis of the protein Tau is of major interest. Indeed, while Tau hyperphosphorylation causes Alzheimer's disease in humans (Noble et al., 2013), this hyperphosphorylation is totally reversible in hibernating species and does not cause brain damage (Su et al., 2008). This reversibility, induced by the activity of phosphatases (Liu et al., 2005), prevents brain cellular damage and probably helps to maintain cognitive function, including spatial recognition, social interaction and predator avoidance, at emergence from a hypometabolic state (**Figure 1**). Nevertheless, it was shown that the activity of these phosphatases is altered during aging (Veeranna et al., 2011, therefore impairing the capacity of Tau dephosphorylation and probably altering the plasticity of the brain of SH with age.

Alteration of Digestive Capacity

In several SH, the decrease in food quantity and quality associated with the unfavorable season leads to changes in gut size and structure (reviewed in Canale and Henry, 2010) to maintain their energy and protein metabolism (**Figure 1**). This reversible gut atrophy leads to additional energetic costs to maintain a functional digestive tract through the unfavorable season (Humphries et al., 2003). In addition, the gut microbiota composition is also flexible, and the intestinal microbial diversity changes in response to food composition (Carey et al., 2013; Stevenson et al., 2014; Amato et al., 2015, 2016; Hatton et al., 2017; Kaczmarek et al., 2017; Carmody et al., 2019; Hauffe and Barelli, 2019). The gut microbiota has been extensively associated with energy homeostasis and metabolic control (Wang et al., 2017), particularly in relation to the effects of microbial metabolites on the gut-brain axis (Clemmensen et al., 2017; Cryan et al., 2019). More precisely, the gut microbiota has a major impact on digestive efficiency and on the nutrients that are rendered available for energy homeostasis (Scheithauer et al., 2016; Riedl et al., 2017). The loss of intestinal microbial diversity has been proven to be detrimental to energy balance and mental health (Cryan et al., 2019). Finally, under a hypometabolic state, protein synthesis is inhibited, and it is known that protein deficiency leads to a decrease in tissue restoration capacity (Humphries et al., 2003). The gut remodeling that operates at each seasonal transition may induce a functional mismatch between food availability and digestive efficiency, therefore impairing metabolic control and cognitive function.

Immunodeficiency

Low body temperature has been linked to reductions in tissue lesions and avoidance of hypoxia during severe systemic inflammation (Liu et al., 2012; Corrigan et al., 2014). Therefore, this dichotomy in host defense might benefit heterotherms during torpor. However, regulated hypometabolism also leads to reduced immune efficiency (**Figure 1**). For example, in several SH, hypometabolism leads to a decrease in the number of circulating leukocytes, a loss of proliferative capacity of lymphocytes and a decrease in the capacity to induce a cellular response (Bouma et al., 2010). In the case of an infection by a pathogen resistant to cold temperatures, organisms in hypothermia would be immunodeficient (Canale and Henry, 2012). Even if the infection triggers a reactivation of metabolic activity, the immune system could be too late to efficiently cope with the infection. Moreover, depending on its severity, infection will lead to either resistance (through microbicidal mechanisms, for example) or tolerance (through management of collateral damage; Medzhitov et al., 2012; Ganeshan et al., 2019; Steiner and Romanovsky, 2019).

CONCLUSION AND PERSPECTIVES IN THE CONTEXT OF GLOBAL WARMING

In this article, we focused on five potential physiological costs of heterothermy, i.e., oxidative stress, excess fat, cognitive defects, digestive inefficiency, and immunodeficiency, which seem the most relevant. Ecological evidence of these costs is lacking, as the benefits of using heterothermy in a constrained, seasonal environment hide these costs. However, captivity annihilates the ecological benefits of using torpor and promotes the emergence of damage, especially during aging. Therefore, experimental studies in photoentrained heterothermic species should be further considered to assess the costs of physiological remodeling at each key phase of seasonal transition (**Figure 1**). Other physiological costs may arise, such as the inhibition of mitosis and protein synthesis during torpor and their rapid reactivation at arousal (Humphries et al., 2003) or imperfect phenotype-environment matching during ontogeny that is costly to compensate once growth is terminated (Auld et al., 2010; Dammhahn et al., 2017).

Understanding how heterothermy comes with ecological and physiological costs for seasonal organisms will provide insights into the constraints and physiological processes underlying the reaction norms of phenotypic performances and fitness components to seasonal environmental change (Boyles et al., 2020). This will help us to better understand physiological processes such as circannual biological rhythms and reproductive cycles (Cayetanot et al., 2005). In addition, such studies may shed light on the mechanisms associated with degrading health conditions at old ages (high survival syndrome of hibernators; Turbill et al., 2011) and better prevent physiological disorders such as obesity (Terrien et al., 2017).

Moreover, understanding the costs of heterothermy will allow us to better understand how seasonality influences the evolution of life cycles, life-history traits, such as senescence, and life-history trade-offs. For example, it is important to know whether these costs affect important fitness components, such as growth or young adult survival and fecundity, or affect only old individuals through faster senescence.

The reasoning in the present perspective paper was focused on circannual metabolic changes, but many of these same changes also occur within days (circadian metabolic changes, i.e., peak oxidative stress during phases of catabolism or arousal from torpor, sleep dept, tau phosphorylation of the central nervous system). Hence, the physiological costs that damage the organism over the long term (seasonal cycles) may actually also operate on a daily basis (daily cycles; Melvin and Andrews, 2009).

Finally, given the major ongoing changes and variability in environmental conditions, the benefits of seasonal metabolic changes may decrease (higher winter temperature, increased winter resource availability). If the costs remain unchanged, the cost/benefit imbalance should reduce the net selective advantage of seasonally flexible genotypes, with a reduction in net immediate survival, reproduction, or growth, or an increase in the aging rate. Costs that are negligible compared to benefits in current environments may become crucial in determining future population dynamics in the case of environmental changes. In this respect, opportunistic heterotherms but not SH should be advantaged by environmental changes (Nowack et al., 2017). Indeed, there might exist a mismatch between unpredictable fluctuations in resource availability that do not follow seasonal patterns and the obligatory physiological remodeling undergone by SH, synchronized on variations in the photoperiod. We know that phenological plasticity can be sex- and reproductive phase-dependent and that different timings of hibernation and reproduction are observed in different populations of arctic ground squirrels (Williams et al., 2012, 2017). However, in the case of environmental changes, in which strong SH are unable to plastically adapt, the gap between seasonal and opportunistic heterotherms might increase, which could alter the adaptive value of being seasonally plastic.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JL, SP, P-YH, and JT contributed to the conception and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Metabolic Regulation, Oxygen Limitation and Heat Tolerance in a Subtidal Marine Gastropod Reveal the Complexity of Predicting Climate Change Vulnerability

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Predictions for climate vulnerability of ectotherms have focused on performance-enhancing physiology, even though an organism's energetic state can also be balanced by lowering resting maintenance costs. Adaptive metabolic depression (hypometabolism) enables animals to endure food scarcity, and physically extreme and variable environmental conditions. Hypometabolism is common in terrestrial and intertidal marine gastropod species, though this physiology and tolerance of environmental change are poorly understood in subtidal benthic gastropods. We investigated oxygen limitation tolerance, hypometabolism and thermal performance in the subtidal, tropical snail *Turritella bacillum*. Survival, cardiac activity and oxygen debt repayment were determined when oxygen uptake was limited by gill function impairment (air exposure) or exposure to hypoxic seawater. Thermal performance and tolerance were assessed from survival and cardiac performance when heated. The ability of snails to regulate metabolism during oxygen limitation was demonstrated by their tolerance of air exposure (>36 h) and hypoxia (>16 h), rhythmicity and reversibility of bradycardia, and inconsistent anaerobic compensation. Under acute heating, mean heart rate was temperature-insensitive in water and temperature-dependent in air. Converging or peaking of individual heart rates during heating suggest maximization of thermal performance at 38–39°C, whereas survival and heartbeat flatlining suggest an upper thermal limit exceeding 42°C. Snails survived 16 h in seawater at 38°C. Their metabolic regulation complies with the oxygen-limiting, sediment-burrowing lifestyle of the species. Although a tropical organism, the species' thermal tolerance so far exceeds present habitat temperatures as to question its susceptibility to centennial climate warming. Our findings reveal the importance of knowing the metabolic regulatory capabilities and conserved physiological attributes of species used in climate vulnerability tests. Studies of ectotherm climate vulnerability that identify generalized trends based on physiologically similar animals may be misleading by missing information on physiological diversity.

Keywords: metabolic depression, hypoxia, air exposure, snail, subtidal, thermal tolerance, climate change

INTRODUCTION

Metabolic rate depression (hypometabolism) is seen in most metazoan groups, especially among animals that hibernate, estivate or undergo diapause (Storey and Storey, 1990; Guppy and Withers, 1999; Marshall et al., 2011). By reducing cellular energy demand, by lowering rates of membrane ion pumping and macromolecule synthesis, less energy is required to achieve a balanced state and energy reserves are conserved (Grieshaber et al., 1994; Hand and Hardewig, 1996; Guppy and Withers, 1999). A lowered cellular energy demand thus enables the endurance of reduced food intake when food is scarce or during behavioral isolation to avoid exposure to extreme and adverse conditions (**Table 1**). It is thus remarkable that a physiological capability that is crucial to surviving environmental uncertainty is missing from mainstream climate vulnerability contexts. Climate vulnerability models for ectotherms focus on the maximization of performance and energy intake relative to temperature, to the exclusion of processes that achieve energetic equilibrium by lowering resting metabolic demand (Brown et al., 2004; Seibel and Drazen, 2007; Sinclair et al., 2016; Huey and Kingsolver, 2019; but for alternatives, see Quévreux and Brose, 2019). To some extent this is understandable as the capability of hypometabolism is limited in many key animal groups, while in others (such as gastropods) its phylogenetic distribution is poorly known. Nevertheless, omitting this aspect of the energy equation challenges the views founded on performance maximization that tropical and marine ectotherms are likely to be especially threatened by climate warming (Deutsch et al., 2008, 2015; Huey et al., 2009; Pinsky et al., 2019).

In gastropod mollusks, metabolic depression likely originated in early marine lineages along with predation avoidance behavior (Palmer, 1979), as withdrawal into the shell occludes respiratory gas exchange. This physiology is crucial to the unique radiation of gastropods in all of Earth's domains (Webb, 2012). By enabling behavioral isolation, limiting environmental contact and reducing evaporative water loss from an organism, hypometabolism underpins the evolutionary transition of gastropods between marine and terrestrial ecosystems, and is a prerequisite for their life on land (Storey and Storey, 1990; Guppy and Withers, 1999; Strong et al., 2008; Webb, 2012). Although, hypometabolism facilitates estivation and prolonged air exposure in intertidal marine snails (sandy beaches, rocky shores, mudflats, and mangroves; Marshall and McQuaid, 2011; Marshall et al., 2011), little is known about its occurrence in benthic, subtidal gastropods (see **Table 1**), which often show significant tolerance of environmental hypoxia (Riedel et al., 2012). The primary environmental triggers of hypometabolism in marine gastropods are reduced oxygen uptake and reduced food intake. Oxygen uptake becomes limited when seawater oxygen tensions decline, and when gill function is impaired during air exposure in intertidal gastropods (**Table 1**). Oxygen limitation is behaviorally induced when snails retract into the shell to avoid unfavorable abiotic exposures (air, salinity, pollution or pH) or during sand burial (McMahon and Russell-Hunter, 1978; Brown and Da Silva, 1979; Little and Stirling, 1984; Kapper and Stickle, 1987; Liu et al., 1990; Marshall and McQuaid, 1993; Marshall et al., 2004, 2011; Marshall, 2009; Proum et al., 2017; **Table 1**). The induction and deactivation of hypometabolism in intertidal gastropods is rapid in order to offset temporal limitations on feeding, which can last from hours to months depending on the tide cycle and season (Marshall and McQuaid, 2011; Monaco et al., 2017). This differs fundamentally from hypometabolism of temperate terrestrial overwintering or diapause animals, which is usually intrinsically programmed and linked to seasonal developmental cycles (Storey, 2002; Storey and Storey, 2004).

Assessing metabolic downregulation often involves following the response of an organism to restricted oxygen uptake, which leads to cellular oxygen limitation and impeded ATP production (**Table 1**). Under such conditions, animals with limited capacity for metabolic depression are obliged to counter the shortfall in aerobically generated ATP through anaerobic production (Stickle et al., 1989; Liu et al., 1990). In animals capable of hypometabolism, anaerobic metabolism only occurs after the lowest depressed metabolic rate is reached (Grieshaber et al., 1994). Anaerobic metabolism can be determined from metabolite accumulation or inferred from an "oxygen debt" that accumulates under hypoxic conditions and is repaid when oxygen becomes available again (Hochachka, 1988). Oxygen debt repayment manifests as a respiratory or cardiac overshoot during recovery in normal oxygen seawater, and its magnitude essentially relates to the degree of anaerobic compensation (Brown and Wynberg, 1987; Hochachka, 1988; Marshall and McQuaid, 1993; Proum et al., 2017). In gastropods, features of cardiac functioning have also been used to assess hypometabolic state. Whereas harmonic bradycardia (regular beat-beat interval) indicates a stable lowered energetic state, intermittent tachycardia or acardia often signifies a respiratory stress response (Marshall and McQuaid, 1993, 1994, 2011; Marshall et al., 2004; Proum et al., 2017). Metabolic regulation is also implied from thermal insensitivity of the metabolic rate (Brown et al., 1978; Marshall and McQuaid, 1994, 2011; McMahon et al., 1995; Marshall et al., 2011; Verberk et al., 2016).

We propose that metabolic depression has critical implications for understanding species' responses to long-term environmental change that are not considered in most predictions of how global warming will affect species (e.g., Brown et al., 2004; Huey and Kingsolver, 2019). To address this, we investigated metabolic depression and thermal tolerance in the tropical subtidal gastropod, *Turritella bacillum* (Turritellidae). By considering a largely unexplored model system, this study adds to understanding of the evolutionary and ecological origins of these traits and helps to evaluate the range of possible responses of gastropods to environmental change. *T. bacillum* is related to other cerithioidean species (Planaxidae, Potamididae, and Thiaridae) that have utilized metabolic depression and estivation to radiate in fringe environments (high-rocky shores, mangroves, and brackish and freshwater systems; Houbrick, 1988; Strong et al., 2008, 2011). Although *T. bacillum* lives in a thermally stable environment (25–31°C), as a species it dates to before the Plio-Pleistocene when temperatures were 2–3°C higher than present (Allmon, 2011; Das et al., 2018); the family dates back to the Cretaceous before the appreciably hotter Paleocene-Eocene Thermal Maximum (PETM, 5°C higher than

TABLE 1 | Behavioral and environmental drivers and hierarchical responses during marine gastropod hypometabolism.

	Behavioral	**Environmental**
	Hypometabolism controlled by the organism in response to a feeding limitation, sand burial or behavioral isolation (withdrawal into the shell)	Hypometabolism associated with internal hypoxia linked to abiotic environmental change, directly or through respiratory incapacity
Drivers	• Food limitation	• **Air exposure** of low intertidal or subtidal gastropods
	• Sand burial	
	• Predator avoidance	• **Hypoxic seawater exposure**
	• Prevent exposure to abiotic extremes (salinity, pH, metal pollution, etc)	• Thermal extreme exposure
Respiratory organ	• Lowered gas exchangeand O_2 uptake is potentially reversible with a change in behavior	• Lowered gas exchange and O_2uptake relates to external conditions or respiratory organ incapacity and is non-reversible while conditions persist
Systemic	• Modulation of cardiac function including transient or sustained bradycardia retains systemic functionality or supports cellular O_2 demand	• Cardiac stress below the internal hypoxia tolerance threshold seen as episodes of tachycardia and/or acardia and cardiac arrest
	• Reduction of blood pO_2 and pH and elevation of blood pCO_2 (isolation)	• Reduction of blood pO_2 and pH and elevation of blood pCO_2 (air) or only reduction of blood pO_2 (environmental hypoxia)
Cellular	• Metabolic downregulation seen by one or combinations of hemolymph reduced pO_2, reduced pH and elevated pCO_2	• Metabolic stressseen by one or combinations of hemolymph reduced pCO_2, reduced pH and elevated pO_2
	• Capable of voluntary temporary reversion of metabolic state to perform limited locomotion	• Reversion to a normal metabolic state that supports growth and reproduction requires normalization of environmental conditions and food uptake
	• Reversion to a normal metabolic state that supports growth and reproduction requires change of behavior and food uptake	

Drivers tested in this study are indicated in bold. See Marshall and McQuaid (1991, 1992a, 1993, 2011) and Marshall et al. (2011).

today; Das et al., 2018; Ivany et al., 2018). Although they are key components of tropical marine ecosystems, information on the physiology of subtidal gastropods is missing from mainstream models for climate change vulnerability (Deutsch et al., 2008; Sunday et al., 2012, 2014; Bennett et al., 2018; Pinsky et al., 2019). The broader objective here was to illustrate that physiological diversity can make predicting the consequences of climate change more complex than appreciated.

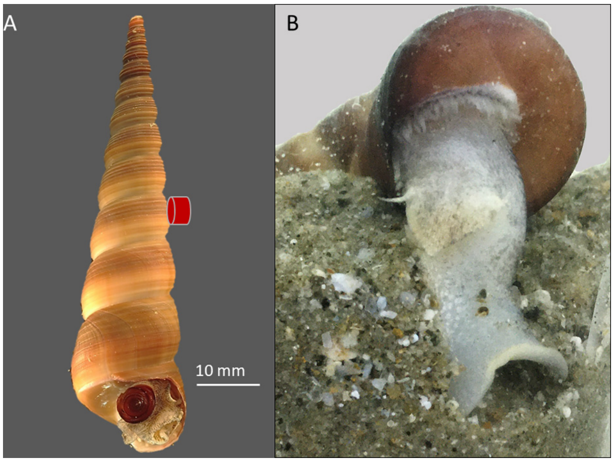

FIGURE 1 | (A) *Turritella bacillum* photgraphed in air, showing retracted head-foot and operculum centrally in the aperture; this is also the assumed posture during sediment feeding. **(B)** Snail burrowing in a glass tank in the laboratory, showing extrusion and probing of the foot. The red symbol indicates the position of the IR sensor used to detect heartbeat.

MATERIALS AND METHODS

Snail Collection and Handling

Turritella bacillum (Kiener, 1843) is widespread in the South China Sea, having a southern equatorial limit in Malaysia and Indonesia and a northern distribution limit in Japan (Scarponi et al., 2018). It is a suspension-feeding subtidal gastropod that remains buried below the sediment surface when not searching for a mate (**Figure 1**; Allmon, 2011; Waite and Allmon, 2016). It occurs down to depths of ~50 m and is reported to tolerate a range of seawater salinities (10–35 psu), temperatures (15–30°C), suspended solids (20 mg L^{-1}) and dissolved oxygen (<5 mg L^{-1}) (Trong et al., 2000; Kwan et al., 2018). The present range of temperatures in Brunei waters is 25–31°C (see **Supplementary Figure 1**; Lane, 2011; Johari and Akhir, 2019).

Snails for the present study were collected from the intertidal zone of a sandy beach at Pantai Tungku, Brunei Darussalam (4.974_N, 114.867_E), between December 2019 and February 2020. These snails had been dislodged from their subtidal habitat during monsoon high sea conditions. Early morning collections of >100 snails were made on several different days. Before experiments, the snails were kept for 1–3 d in glass tanks of recirculating water [60 cm (L) × 30 cm (W) × 40 cm (H); Eheim Professional pumps] at 27°C, 33 psu salinity and without the provision of planktonic food. Their survival in the laboratory was excellent, with only a two mortalities over 12 weeks. Snails provided with beach sediment rapidly buried themselves (**Figure 1**), remaining buried without contact with the overlying water for over 6 weeks. Snails not provided with sediment remained sedentary, though a few showed heave-crawling along the tank surface. When not moving, the head-foot and operculum were usually located centrally at the aperture (**Figure 1**). In some, the body was withdrawn into the shell, sometimes to near the third from last whorl. Although the small operculum does not completely cover the shell aperture, it minimizes contact of the body with the outside environment and prevents sediment intrusion when deeper in the shell. Retracted snails invariably re-emerged at the shell aperture within 30 min of exposure in air (24°C; benchtop temperature). Snails that were not sacrificed to determine tissue mass, were returned to the subtidal sea near to where they were collected.

Oxygen Limitation: Tolerance and Metabolic Depression

We assessed survival and physiological responses of snails to restricted oxygen uptake through exposure to either air, which limits oxygen uptake at the gills, or hypoxic seawater (**Table 1**, Proum et al., 2017). Hypoxia leads only to lowered hemolymph PO_2 while air exposure also causes elevated hypercapnia and potentially reduces hemolymph and cellular pH. In land snails, lowered hemolymph pH due to elevated hemolymph PCO_2, when gas exchange is reduced during withdrawal into the shell, is the primary driver of metabolic downregulation (Barnhart and McMahon, 1988).

Survival of Oxygen Limitation

Tolerance of air exposure was determined for snails held in loose polythene bags in a Memmert Peltier-cooled (IPP400) incubator for 16 (n = 12), 24 (n = 12), 36 (n = 5) and 48 h (n = 6) at 27 ± 0.2°C and 95% R.H. (monitored using DS1923-F Hygrochron I-buttons). Survival of hypoxic seawater was determined for snails (n = 5) exposed for 16 h to 1.0–1.5 mg O_2 L^{-1} (27 ± 0.2°C). Hypoxic conditions were created by bubbling mixtures of air and N_2 gas into seawater (33 psu) in a 5-L beaker, held inside a Grant waterbath at 27 ± 0.2°C. The oxygen concentration (% Air Saturation) of the water was continuously monitored using a Witrox fiber-optic system (Loligo Systems, Denmark). In the present experiment, snails were retained in respiratory chambers (~27 mL volume) inside the beaker, while the oxygen concentration of the water was lowered from ambient to 1.5 mg L^{-1} over 15 min. At 1.5 mg L^{-1} O_2, the respiratory chambers were capped and left overnight for 16 h in the waterbath, allowing the snails to extract further oxygen from the chamber water. The oxygen concentrations of water in the chambers containing snails decreased by an average of 0.5 mg L^{-1} O_2 during this period. After the 16 h period, survival was determined by the re-emergence of snails when exposed to air at 24°C (see above).

Determining Metabolic Regulation From Cardiac Activity

We monitored cardiac activity in snails exposed to air or hypoxic seawater. In air, cardiograms were collected over 16 h for four snails kept in loose polythene bags in a Memmert Peltier-cooled (IPP400) incubator (27 ± 0.2°C; 95% R.H.; DS1923-F Hygrochron I-buttons), and for hypoxic seawater exposure, cardiograms were collected for a single snail during the oxygen debt formation experiment (see below; 3 h, 2.02–1.3 mg L^{-1} O_2). Heartbeats were detected with IR-sensors fitted to the outside of shells at the third from last whorl (**Figure 1**), and coupled to bridge amplifiers and a Powerlab system (ADInstruments, Australia; Marshall et al., 2011). Under normal circumstances, the cardiovascular system of gastropods functions to deliver oxygen to the cells (Marshall and McQuaid, 1992b). Unlike ciliary ventilation of the mantle cavity, perfusion [heart rate (HR) and stroke volume] in gastropods can be dynamically modulated (within minutes) in response to cellular oxygen demand, consequently it reflects demand as well as oxygen uptake and delivery (Marshall and McQuaid, 1992b, 2011; Marshall et al., 2004, 2011). From cardiogram traces and HR-time plots, we checked for features that typically indicate metabolic regulation, such as harmonic bradycardia (similarity of beat-to-beat variation) and the recoverability from this bradycardia. Capacity-limited, stressed animals do not recover their heartbeat rhythmicity, but instead show tachycardia interspersed with acardia (see section "Introduction"; Marshall and McQuaid, 1993, 2011).

Determining Metabolic Regulation From Oxygen-Debt Formation

The formation of an oxygen debt was assessed for individual snails by comparing the aquatic respiratory rate in normal seawater *before* and *after* exposure to air or hypoxic seawater. An overshoot in the recovery (*after*) respiratory rate was taken to indicate repayment of an oxygen debt formed under oxygen-limited conditions, with the degree of overshoot indicating the level of incapacity for total metabolic depression (see section "Introduction"). For the air exposure treatment, snails (n = 12) were held in air for 16 h, inside open respiratory chambers contained in open polythene bags [Memmert Peltier-cooled (IPP400) incubator; 27 ± 2°C (95% R.H)]. Two hypoxic seawater exposure treatments were used; snails were exposed either to 2.02–1.3 mg L^{-1} O_2 for 3 h (treatment 1; n = 8) or to 1.5–1.0 mg L^{-1} O_2 for 16 h (treatment 2; n = 4). The oxygen concentration of seawater contained in a 5-L beaker inside a Grant waterbath set to 27 ± 0.2°C was manually lowered (see above, "survival of oxygen limitation"). In treatment 1, the water was lowered from normoxia at 6.73 to 2.02 mg L^{-1} (over 10 min), kept stable at 2.02 ± mg L^{-1} (for 50 min) and then lowered further to 1.3 mg L^{-1} (for 100 min), giving a total hypoxia exposure of 160 min. Treatment 2 followed the same protocol as described under "survival of oxygen limitation," giving an exposure of 1.5–1.0 mg L^{-1} O_2 for 16 h.

In the *before* respiratory rate determinations, snails were left to settle in open respiratory chambers (25-27 mL water volume, excluding snails) in recirculating seawater for 30 min (27°C). The chamber lids were then secured underwater and the chambers containing snails were rapidly transferred to a waterbath (Lab Companion RW-0525P, Korea). Snails were allowed to extract oxygen from the chamber water for precisely determined periods of ~20 min, ensuring a reduction in the chamber O_2 concentration that was detectable, but did not fall below 65% Air Saturation. In the *after* respiratory rate determinations, snails were given a shorter time to recover in seawater (10 min) in order to avoid missing a possible oxygen debt repayment (see Proum et al., 2017). The oxygen uptake incubation temperature for the air treatment was 27 ± 0.1°C and that for hypoxia seawater treatment was 30 ± 0.1°C; the latter was tweaked retrospectively to achieve a greater O_2 extraction rate from the chamber water.

Oxygen concentration (% Air Saturation) was measured using a Witrox system (Loligo Systems, Denmark), comprising optical spot sensors glued inside each individual respiratory chamber and fiber-optic cables adhered to the outside of the chambers. After incubation, the snails were removed from their chambers and the oxygen concentration of the stirred water (magnetic bead) was logged every second until the readings stabilized (~2 min) at temperatures of 26–27°C. For each set of measurements, oxygen concentrations were also determined for two control chambers lacking snails. Snail oxygen uptake was determined from the difference between the oxygen concentration in a chamber containing a snail and that of the average for the control chambers. Percentage air saturation values were converted to μmol L^{-1} O_2 and then to μmol O_2 h^{-1} g^{-1} wet tissue mass, after cracking open the shells and removing and weighing the tissue. The chamber water volume excluded the volume of the snail. Mass-specific *before* and *after* respiratory rates were compared with dependent *t*-tests, using Statistica v.12. All data exhibited homogeneity of variances (Levene's test). Because of the narrow mass range of the snails used in each experiment, linear relationships for oxygen uptake versus mass were not significant (p = 0.78–0.89), precluding mass-scaling of respiratory rates. Mean snail wet masses (± SD) were 0.89 ± 0.2 g in air, 0.78 ± 0.16 g in hypoxia treatment 1, and 1.78 ± 0.1 g in hypoxia treatment 2.

In a separate experiment, we determined oxygen uptake during air (16 h) and hypoxia exposure (treatment 2, 16 h) for individual snails by measuring the oxygen concentration in closed chambers. Mass-specific oxygen uptake rates were extremely low in either medium; those in air (mean ± SD, 0.046 ± 0.008 μmol O_2 h^{-1} g^{-1}, wet mass = 1.26 ± 0.196 g, n = 6) were ~3 times greater than those in hypoxic seawater (0.014 ± 0.004 μmol O_2 h^{-1} g^{-1}, wet mass = 1.79 ± 0.103 g, n = 4).

Thermal Performance

Survival and Cardiac Performance During Acute Heat Exposure

Cardiac activity was monitored in snails that were heated in air (n = 7) or water (n = 10) from 30°C at 1°C for 10 $\min^{-1}$. Snails, previously fitted with IR-sensors, were retained in plastic bags (air) or 50 mL beakers containing aerated seawater (33 psu), inside a programmable temperature bath (Grant TXF200, Cambridge, United Kingdom). Heating started after 10 min equilibration at 29–30°C. Body temperature was determined from calibrated K-type thermocouples held next to the snails and connected to a TC-08 Picolog interface (Pico Technology, Cambridge, United Kingdom). Temperature and HR, measured in beats per minute (BPM), were logged simultaneously every 1 min. The HRs of individual snails were then averaged for every 1°C, and these values were used to determine mean HR for all snails for each temperature. The effect of temperature on HR for snails in air or water was determined using Friedman ANOVAs for multiple dependent samples (Statistica v.12, StatSoft, New York, United States).

Survival of acute heating was determined for snails (n = 17) heated in water from 30°C, after 10 min equilibration, to either 39, 41, 43, or 45°C at a rate of 1°C for 10 $\min^{-1}$, a rate that ensured thermal equilibration and physiological adjustment to each 1°C rise. Survival was ascertained by the re-emergence of snails at the shell aperture in air (24°C; see above), immediately after the heat treatment, and again after 24 h recovery in aquaria at 27°C. In cases where snails did not re-emerge, shells were cracked open to confirm mortality.

Survival of Chronic Heat Exposure

Although *T. bacillum* snails likely naturally experience only slight temperature variation (either daily or seasonally) during their lifetimes, acute heating experiments nonetheless inform about physiological capacity limitation, and thus are useful in comparisons between species (Marshall et al., 2011; Monaco et al., 2017). Acute heating protocols, irrespective of the habitat thermal regime, are also widely used to determine *thermal safety margins* and *warming tolerance* in studies assessing the climate vulnerability of animals (see Deutsch et al., 2008; Sinclair et al., 2016; Pinsky et al., 2019). To improve our understanding of the ability of these snails to tolerate temperatures experienced naturally, we assessed chronic heat exposure responses. Survival was determined for snails (n = 84) in air or water, kept at constant temperatures of 27, 33, 36, 38, and 40°C for 16 h (overnight). The number of snails surviving after each treatment was determined as above, immediately after the heat treatment, and again after 24 h recovery in seawater aquaria at 27°C.

RESULTS

Oxygen Limitation Tolerance and Metabolic Depression

Survival and Cardiac Activity

All snails (100%) survived air exposure for up to 36 h, though 100% mortality occurred during 48 h exposure. A total of 100% survival was also observed after 16 h hypoxia exposure. In the cardiac activity experiments, all four snails showed HR recoverability during the 16 h air exposure period. The HR varied between ~10 and 35 BPM, and there were clear episodes of recovery from bradycardia (<15 BPM), including after ~13 h of air exposure (p = 0.0039; the probability of recovery occurring 3–4 times, compared to 0, 1, or 2 times, in each of four trails). The periodicity of bradycardia varied among individuals, with two showing evenly spaced episodes (**Figure 2**, bottom two panels). Heartbeat rhythmicity (beat–beat periodicity) was maintained for the entire exposure period, for faster or slower HRs (**Figure 3**). Similar heartbeat patterns were observed in the tracer snail exposed to hypoxic seawater. Bradycardia in

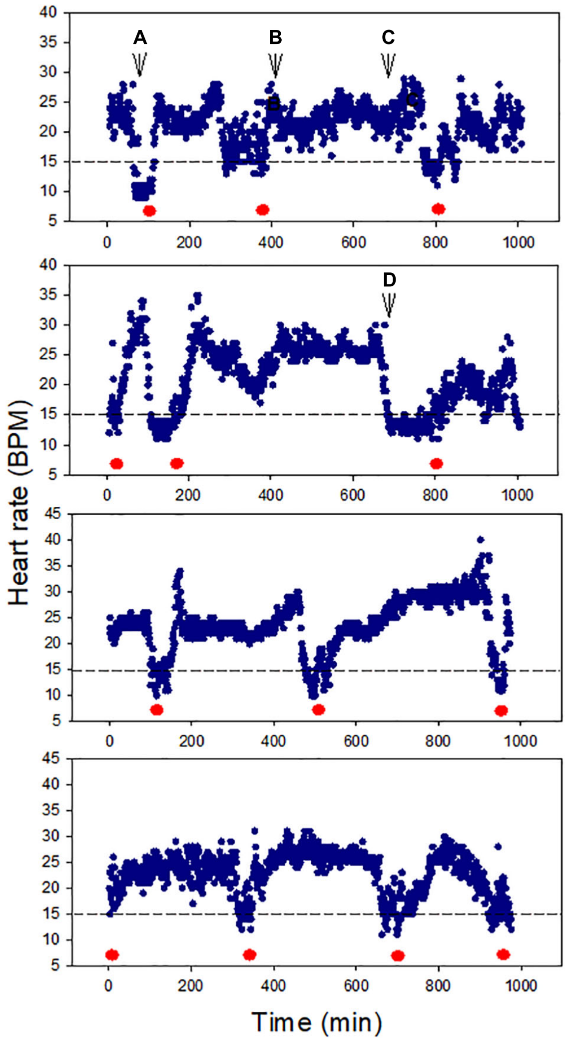

FIGURE 2 | Heart rate (HR) modulation in four snails during 16 h air exposure (27 ± 0.2°C). All snails showed distinct cycles of depression and elevation of heart rate, including bradycardia (defined here as 15 BPM, red markers). Harmonic heart beating was observed throughout the 16 h period. Letters pinpoint where cardiograms shown in **Figure 3** were collected.

this snail was characterized by sustained harmonic beating (**Supplementary Figure 2**).

Oxygen Debt Formation

We found no evidence of oxygen debt formation in snails during air exposure (16 h). Although the mean after-exposure respiratory rate was greater than the before-exposure rate, the difference was not statistically significant [mean ± S.E.; 3.99 ± 0.63 and 4.5 ± 0.63 μmol h^{-1} g^{-1} wet mass ($t = -1.22$; $p = 0.25$; **Figure 4**]. Individual rates ranged between 1.1 and 8.6 and 1.9 and 9.8 μmol h^{-1} g^{-1} wet mass, for before- and after-exposure, respectively (**Figure 4**). In some cases the after-exposure respiratory rate increased whereas in others it decreased. Hypoxia exposure led to clear oxygen debt formation, with significant elevation of the mean after-exposure oxygen uptake rate. In experiment 1 (3 h), the mean values (± S.E.) were 5.19 ± 0.71 for before, and 8.27 ± 1.24 μmol h^{-1} g^{-1} wet mass, for after exposure ($t = -3.91$, $p = 0.005$; **Figure 4**). Individual rates varied between 2.1 and 7.5 for before and 1.9 and 13.7 μmol h^{-1} g^{-1} wet mass for after exposure. In only one case was the after-exposure rate not elevated (**Figure 4**). A similar result was obtained in experiment 2 (16 h). Mean values (± S.E.) were 4.28 ± 0.51 for before and 8.55 ± 1.59 μmol h^{-1} g^{-1} wet mass, for after exposure ($t = -3.24$, $p = 0.048$; **Figure 4**). Individual rates ranged between 3.1–5.5 and 4.1–12.6 μmol h^{-1} g^{-1} wet mass, respectively, for before- and after-exposure treatments.

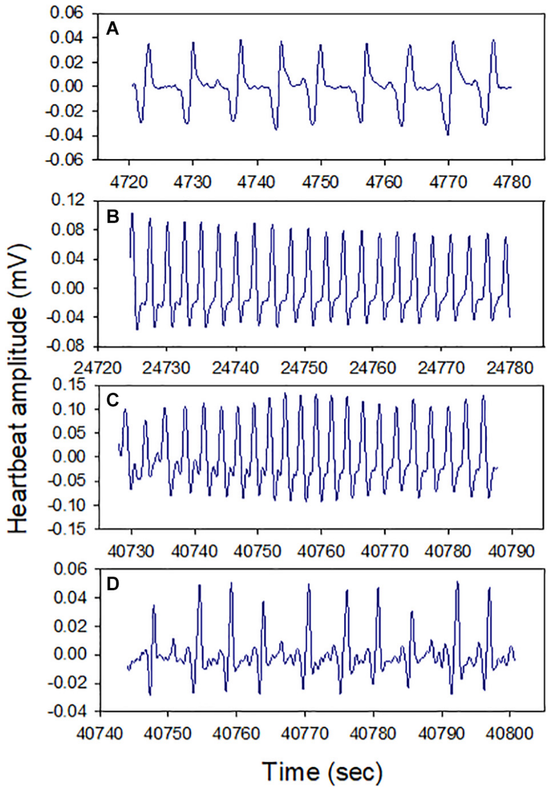

FIGURE 3 | Cardiograms of snails at different times during 16 h air exposure (Letters **A–D** refer to points indicated in **Figure 2**). Heartbeat rhythmicity (beat to beat periodicity) was maintained throughout this exposure period in all snails.

Thermal Performance and Tolerance

Cardiac performance under acute warming differed between snails in air and water. In water, mean HR was thermally insensitive, with little variation across the entire range of temperatures tested (24.1–31.6 BPM; 29–41°C; χ^2 (df = 12) = 20.12, $p = 0.065$; **Figure 5**). Variation among individual HRs was high (averages ranged between 21.8 and 44.6 BPM), and HRs were largely thermally insensitive, but converged at ∼38°C (**Figure 5**). In contrast, HRs were temperature sensitive in air, increasing with heating up to 39°C and then decreasing [χ^2 (df = 11) = 56.25, $p < 0.001$; **Figure 5**].

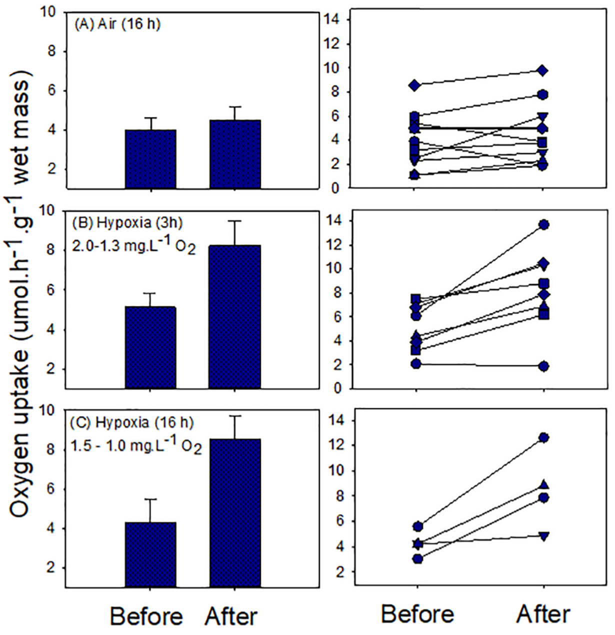

FIGURE 4 | Oxygen uptake in normal seawater before and after exposure to air or hypoxic seawater. Left panels show means ± S.E. values and right panels show individual snail responses.

Flatlining occurred at or above 42°C. In air, mean HRs varied between 22 BPM at 30°C and 45 BPM at 39°C. Variability among individuals increased above 31°C, reflecting variability in their thermal sensitivities (note SD values and slopes in **Figure 5**).

Snails survived acute heat ramping in water up to 43°C (**Table 2**). The chronic thermal exposure experiments showed complete survival of snails in air or water up to a constant 36°C (16 h; **Table 2**). Whereas all snails survived exposure to 36°C when tested immediately after the experiment, none survived after 24 h (27°C, seawater) and no snails survived 40°C exposure (**Table 2**).

DISCUSSION

Oxygen Limitation and Metabolic Depression

We show attributes of *T. bacillum* that indicate metabolic regulation rather than stress-related responses to oxygen uptake limitation. Metabolic downregulation is seen by (1) the suppression and recovery of HR, (2) rhythmic (harmonic) bradycardia, and (3) the absence of an oxygen debt when snails were exposed to air or hypoxic seawater. The responses of animals with little capability to regulate metabolism under oxygen limitation include a loss of heartbeat rhythmicity, long or continuous acardia, no recovery of HR, and an accumulation of an oxygen debt (Marshall and McQuaid, 1991, 1992a,b, 1993, 1994; Marshall et al., 2004; Proum et al., 2017). Although reduced cardiac activity often signals metabolic depression (Marshall and McQuaid, 1991, 1993, 2011; Marshall et al., 2004), we could not reconcile the HRs of *T. bacillum* snails with their low oxygen uptake rates in air or hypoxic seawater. Under these circumstances, the heart likely functions to maintain hemolymph circulation. In intertidal gastropods, HR relates to the rate of oxygen uptake in air and seawater when rates are varied by heating, but this relationship varies between the media due to impeded oxygen uptake in air-exposed snails (Marshall and McQuaid, 1993).

An oxygen debt reflects anaerobic metabolic compensation when aerobic ATP generation is reduced, and indicates an organism's ability to downregulate overall metabolism (Hochachka, 1988; Grieshaber et al., 1994). A limited ability for downregulation corresponds with significant anaerobic compensation under declining cellular oxygen levels. The absence of or an inconsistent or weak oxygen debt formed in *T. bacillum* complies with observations of some intertidal gastropods (Brown and Wynberg, 1987; Marshall and McQuaid, 1993, 1994; Proum et al., 2017). Our data suggest a possible critical PO_2 (the environmental O_2 level at which ATP generation is largely anaerobic) for *T. bacillum* of between 2.2 and 1.5 mg L^{-1} O_2. This was suggested by an hypoxia-induced oxygen-debt, and by the HR pattern of a tracer snail under hypoxia that recovered from bradycardia at 2.2 mg L^{-1} O_2 seawater, but not at lower oxygen concentrations (<1.5 mg L^{-1} O_2). Striking individual differences were seen in oxygen debt formation during hypoxia, implying different energetic requirements of individual snails. Some individuals may need to complete energy-demanding, intrisically fixed stages of digestion, reproduction and growth (requiring anaerobic compensation), before reverting to an adaptive energy-conserving hypometabolic state (see Marshall et al., 2004; Marshall and McQuaid, 2011).

Metabolic regulation characterizes land-dwelling and intertidal gastropods, but is poorly reported in subtidal gastropods, despite their often exceptional tolerance of hypoxia (Stickle et al., 1989; Liu et al., 1990; Guppy and Withers, 1999; Sokolova and Pörtner, 2001, 2003; Storey, 2002; Marshall and McQuaid, 2011; Marshall et al., 2011; Riedel et al., 2012, 2014). By facilitating tolerance of environmental stress and food shortages, regulation underpins the exceptional evolutionary transitions and radiations by gastropods (Webb, 2012). A hypometabolic capability should benefit *T. bacillum* by allowing it to withstand restrictions on food and oxygen supply to the cells. Environmental hypoxia occurs widely in oceanic environments, especially in association with upwelling, and is expected to worsen as coastal eutrophication and global warming progress (Vaquer-Sunyer and Duarte, 2008; Riedel et al., 2012). Hypoxic conditions can also be induced behaviorally in sediment-dwelling and burrowing marine gastropods (Brown and Da Silva, 1979; Marshall, 2009; Proum et al., 2017). *T. bacillum* snails survived in the laboratory for weeks without food or when completely buried with no contact with the above-sediment water (see section "Materials and Methods"). These snails should experience natural disruptions in food supply when buried or when feeding is curtailed by high suspended sediment loads (Ellis et al., 2002; Lohrer et al., 2004). The observation of metabolic depression during air exposure, however, does not

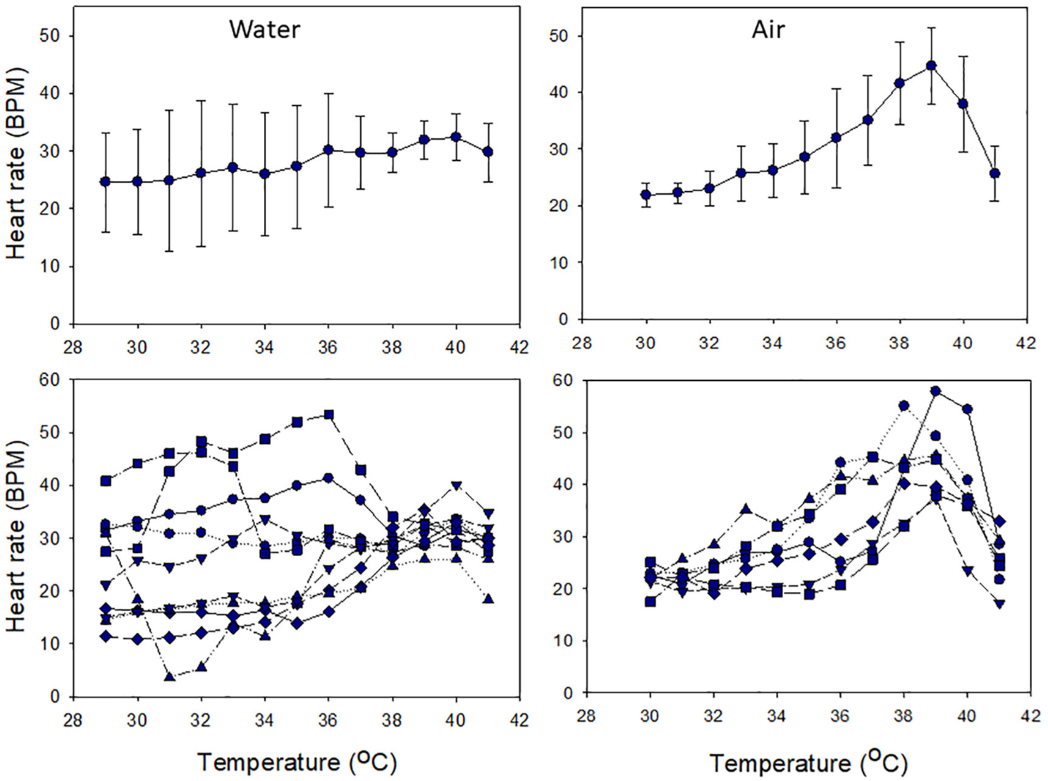

FIGURE 5 | Heart rate responses of snails to heating in air or water from ∼29°C over 3 h. Upper panels show mean values (± S.D.), and lower panels show individual responses.

TABLE 2 | Survival of heat exposure of *T. bacillum*.

Temp (°C)	Duration/medium	Survival% end (alive/total)	Survival% 24 h* (alive/total)
Acute**			
39	Ramping < 2 h/water	100 (4/4)	100 (5/5)
41	Ramping = 2 h/water	100 (5/5)	100 (5/5)
43	**Ramping > 2 h/water**	**100 (4/4)**	75 (3/4)
45	Ramping > 2 h/water	0 (0/4)	0 (0/4)
Chronic			
27	16 h/air	100 (12/12)	100 (12/12)
36	**16 h/air**	**75 (9/12)**	75 (9/12)
27	16 h/water	100 (12/12)	100 (12/12)
36	16 h/water	100 (12/12)	100 (12/12)
38	**16 h/water**	**100 (5/5)**	0 (0/5)
40	16 h/water	0 (0/5)	0 (0/5)

**Survival was assessed from snail responding to air exposure and the appearance of the operculum in aperture at the end of each thermal exposure and 24 h later (27°C, 33 ppt). **Ramp rate was 1°C for 10 min^{-1}. Bold indicates highest temperature at which 50–100% of the population survived.*

necessarily mean an evolutionary association with the intertidal ecosystem, but rather likely represents a general physiological response to reduced oxygen uptake (see below).

Thermal Performance and Tolerance

Whereas tolerance of air exposure can be explained by the capacity of *T. bacillum* for hypometabolism and hypoxia tolerance, its heat tolerance and the difference between aerial and aquatic thermal performance were unexpected. Because oxygen uptake is not inhibited in normoxic seawater, we assume that the thermal insensitivity of cardiac activity in snails reflects temperature-independent metabolism (Marshall and McQuaid, 1993; Verberk et al., 2016), which corresponds with the thermal stability of the habitat of these snails (25–31°C; **Supplementary Figure 1**). However, because oxygen uptake is severely limited in air, the observed thermal dependence of aerial HR indicates uncoupling of cardiac and metabolic performance. Despite this lack of functional association, the aerial cardiac performance of *T. bacillum* was not random and was clearly maximized at 39°C. The high individual variability in HR and in thermal sensitivity again likely refers to different energy demands and stable states of individuals (see above). The initially variable HRs in water converged at 38°C, and variably sensitive individual rates in air-exposed snails peaked at 39°C. Together, these data suggest a physiological threshold, with optimal thermal performance (T_{opt}) at 38–39°C. Flatlining of HR under acute heating indicates an upper thermal limit and the onset of mortality at ∼42°C (Stenseng et al., 2005; Polgar et al., 2015). This was confirmed by the highest temperature for 100% survival under acute ramping of 43°C.

It is difficult to ascribe adaptive significance to the observed thermal performance of *T. bacillum* given the habitat temperatures it naturally experiences. Its thermal physiology suggests trait conservation, with unexpressed phenotypes under natural circumstances being retained in evolutionary lineages (Angilletta, 2009; Grigg and Buckley, 2013). Although *T. bacillum* is thought to have originated since the Plio-Pleistocene period (Das et al., 2018), older turriteline lineages date to the Cretaceous and have survived the Paleocene-Eocene Thermal Maximum (see Ivany et al., 2018), the hottest time in the history of the living planet. Alternatively, heat tolerance may be a more recent acquisition, linked to earlier occupation of the thermally variable intertidal zone. The closest generic relative of *T. bacillum* is an intertidal snail (*Batillaria*; Strong et al., 2011) and its cardiac activity (in air) bears similarity to that of low-shore species, *Turbo bruneus* and *Trochus radiatus* (Monaco et al., 2017). Phylogenetic conservatism of thermal tolerance in niche-radiating gastropods is nicely exemplified by the gross mismatch between habitat temperature and thermal physiology in an equatorial freshwater snail (*Clea nigricans*), belonging to a predominantly marine and intertidal clade (Buccinoidea; Polgar et al., 2015). Disentangling these possible interpretations requires a solid trait-based phylogeny.

Climate Vulnerability and Physiological Diversity

Our findings exemplify the difficulty of making general predictions for climate vulnerability in marine ectotherms. Such predictions are commonly founded on relationships between species' thermal tolerances, habitat temperature conditions, and expected long-term environmental warming rates. For benthic animals these inferences are not straightforward, as species thermal limits can be flexible, can vary with experimental heating rates, and rates of ocean warming can vary spatially. Nonetheless, a *warming tolerance* metric calculated as the difference between a species' upper thermal limit and maximum habitat temperature, provides some insight into the probability of species persistence. The broadscale application of this approach across latitudinal gradients suggests that tropical species are particularly vulnerable to a predicted 2–3°C increase in centennial temperature (Deutsch et al., 2008; Huey et al., 2009). In the case of *T. bacillum*, however, a *warming tolerance* of 7°C, based on our most conservative estimates of heat tolerance (100% survival for 16 h at 38°C) and mean maximum habitat temperature of 31°C (**Supplementary Figure 1**), far exceeds this general prediction. We recognize the importance of chronic heating to climate vulnerability in marine subtidal animals (Peck et al., 2009; Richard et al., 2012), but note that tolerance of *T. bacillum* of acute heat ramping (100% survival to 43°C) is greater than that recorded for other tropical subtidal gastropods (e.g., 41.1–42.4°C; Nguyen et al., 2011). We are also cognizant of the fact that past extinctions of turritelline snails are ascribed to changes in global temperature, suggesting warming vulnerability of the group in general, but not necessarily of specific cases (Anderson and Allmon, 2020).

The mechanisms underlying the persistence of subtidal marine populations and species over temperature changes spanning decades are complex and likely to relate to organismal energetics, rather than thermal tolerance limits. Current models based on 'fixed' energy metabolism of individuals imply that survival at higher temperatures requires an increase in energy intake (food) to meet thermally elevated energy demands (Brown et al., 2004; Van der Meer, 2006; Sinclair et al., 2016; Huey and Kingsolver, 2019). Such elevated energy demands occur at the same time that performance becomes suboptimal and food availability is likely limited by reduced production and generally a reduced energy flow through the ecosystem (see Morais et al., 2020). However, these energetic constraints can be negated or minimized in animals with flexible or temperature independent metabolic rates that allow the lowering of resting energy demands, as occurs in many gastropods, including *T. bacillum*.

Accounting for metabolic regulation where this physiology exists, rather than assuming "fixed" metabolism, is crucial to predicting responses to climate warming. The capacity for metabolic regulation appears to exist in marine "pulmonates" and caenogastropod groups (Buccinoidea, Littorinoidea, Muricoidea, and Cerithioidea), while seems less well developed in the Patellogastropoda and the Vetigastropoda (McMahon and Russell-Hunter, 1977; Kapper and Stickle, 1987; Liu et al., 1990; Marshall and McQuaid, 1991, 1992a,b, 1993, 1994; Riedel et al., 2012; Leung et al., 2017). *T. bacillum* belongs to the Cerithoidea, a group that has radiated in physically variable and extreme fringe environments, including rocky-shores, mangroves, estuaries and brackwater streams. Although no phylogenetic data are available to confirm precedence of hypometabolic physiology in subtidal cerithioideans (Strong et al., 2011), exaptation of this trait in fringe lineages is suggested by the antiquity of turritelline snails (Marshall and McQuaid, 1991).

CONCLUSION

Turritella bacillum exhibited physiological features consistent with metabolic regulation and heat tolerance. Metabolic depression sustains environmental isolation, prolonged starvation and tolerance of hypoxia, which enable animals to withstand environmental deterioration, and which should provide resilience against climatic-driven environmental change. Though common in marine gastropods, hypometabolism is poorly integrated into contemporary hypotheses concerning responses to climate change, which center on the optimization of energy intake and "fixed" metabolic rates (Van der Meer, 2006). The general hypothesis that tropical marine animals are especially vulnerable to climate warming (Deutsch et al., 2008; Tewksbury et al., 2008; Sunday et al., 2014; Pinsky et al., 2019) is not supported by our findings. Attempts to predict species responses to climate change would benefit from considering greater physiological diversity (Somero, 2010), rather than focusing on increasing the count of physiologically similar species. Identifying and including information for taxa that show metabolic regulatory physiology should enhance the accuracy of climate vulnerability predictions.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

DM performed the research. DM and CM formulated the concept and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.01106/full#supplementary-material

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