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Primate hibernation: The past, present, and promise of captive dwarf lemurs

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Abstract

The dwarf lemurs (*Cheirogaleus* spp.) of Madagascar are the only obligate hibernators among primates. Despite century-old field accounts of seasonal lethargy, and more recent evidence of hibernation in the western fat-tailed dwarf lemur (*Cheirogaleus medius*), inducing hibernation in captivity remained elusive for decades. This included the Duke Lemur Center (DLC), which maintains fat-tailed dwarf lemurs and has produced sporadic research on reproduction and metabolism. With cumulative knowledge from the field, a newly robust colony, and better infrastructure, we recently induced hibernation in DLC dwarf lemurs. We describe two follow-up experiments in subsequent years. First, we show that dwarf lemurs under stable cold conditions (13°C) with available food continued to eat daily, expressed shallower and shorter torpor bouts, and had a modified gut microbiome compared to peers without food. Second, we demonstrate that dwarf lemurs under fluctuating temperatures (12–30°C) can passively rewarm daily, which was associated with altered patterns of fat depletion and reduced oxidative stress. Despite the limitations of working with endangered primates, we highlight the promise of studying hibernation in captive dwarf lemurs. Follow-up studies on genomics and epigenetics, metabolism, and endocrinology could have relevance across multidisciplinary fields, from biomedicine to evolutionary biology, and conservation.

KEYWORDS
Cheirogaleus, Duke Lemur Center, heterothermy, torpor, tropical

INTRODUCTION

The dwarf lemurs of Madagascar (*Cheirogaleus* spp.) are well known today as the only obligate hibernators among primates. Individuals from all known species living across diverse habitats in Madagascar hibernate in the wild,[1,2] which is supported by scientific evidence from a series of sporadic waves of research over the past century, with significant lag times in between. Scientific studies first documented seasonal fattening and lethargy in small and nocturnal Malagasy primates in the early 20th century at a time when the exotic and strange fauna from Madagascar was making news in the Western scientific world.[3,4] By the 1950s, a handful of individual lemurs were in captivity in France, providing some of the first quasi-experimental evidence of metabolic depression in lemurs. These studies, conducted on a few individuals, confirmed field anecdotes that mouse (*Microcebus* spp.) and dwarf lemurs can express torpor.[5,6] In captivity, the lemurs deposited significant fat stores, particularly in their tails, before falling into a lethargic period characterized by reduced body temperature, food intake, and overall activity.[6,7] Malagasy understanding of dwarf lemur habits corroborated this early scientific knowledge. The Malagasy name for dwarf lemurs, *matavy-rambo*, literally translates to "fat tail," and human communities from, for example, eastern Madagascar have reported

lemurs to be absent from the forest during the dry season, perhaps hiding underground or inside rotten trunks.[7]

In the 1970s and 1980s, breeding colonies of dwarf and mouse lemurs were established in France and the United States and yielded new insights. A series of experiments sought to clarify the effects of between seasonality, photoperiod, temperature, and food availability on lemur reproduction and metabolism.[7–12] This body of work established that both mouse and dwarf lemurs are strongly seasonal breeders[8,13,14] and display cycles of body-mass gain and loss even when maintained under constant photoperiod and temperature conditions.[12] Nevertheless, because evidence of hibernation in the wild was still anecdotal, husbandry conditions in captivity (e.g., warm room temperatures and abundant food) were not established to accommodate hibernation.[10,14] Understanding lemurs' metabolic flexibility—essential for hibernation—remained limited until new data emerged two decades later.

Fieldwork conducted around the turn of the 21st century on the feeding ecology and ecophysiology of hibernation in the fat-tailed dwarf lemur (*Cheirogaleus medius*), one of nine dwarf lemur species, sparked renewed interest among the scientific community.[15–18] New field data clarified that fat-tailed dwarf lemurs are obligate hibernators in the wild, which differentiated them from mouse lemurs that are now classified as facultative heterotherms.[19] In Madagascar, the fat-tailed dwarf lemur inhabits western dry deciduous forests[20] where food availability is particularly seasonal. To combat resource scarcity, fat-tailed dwarf lemurs hibernate during the dry season. Prior to hibernation, they build up lipid stores by shifting their diets to primarily feed on ripe fruits.[18] For up to 7 months per year, they curl up inside tree holes and live off their lipid stores. Field researchers found that dwarf lemurs hibernating under stable ambient conditions show the classic patterns of temperate hibernators: They cycle between prolonged bouts of metabolic depression when body temperatures approximate that of the ambient environment and short arousals when body temperatures achieve euthermia.[15,16] Curiously, these data highlighted that under variable ambient temperature conditions, fat-tailed dwarf lemurs continue to thermoconform.[15,16] This is in contrast to thermal conditions experienced by many temperate hibernators that generally occupy stable cold hibernacula during the winter.[21] For dwarf lemurs that passively rewarm during hot middays and that can minimally achieve 30°C, arousals are dispensable.[15,16] That dwarf lemurs can express torpor–arousal cycles or thermoconform under variable temperature conditions without arousals is perhaps a moot point to the lemurs themselves as these two hibernation styles are energetically equivalent[17]—although perhaps not physiologically so. Indeed, a long-standing view in hibernation research acknowledges that periodic arousals from torpor bouts at low temperatures incur cellular damage, such as oxidative stress.[22]

Importantly, the ability to passively rewarm during torpor to reduce costs of arousal[17] is not exclusive to dwarf lemurs, and several other tropical hibernators are now known to use this strategy, including tenrecs,[23,24] elephant shrews,[25] mouse lemurs,[26] and many other mammals and birds.[27,28] Taken together, this emerging body of literature on the flexibility inherent to the tropics challenges traditional definitions based on classic hibernators from temperate zones, such as squirrels and marmots.[29] Such metabolic flexibility even raises key questions about the very nature of hibernation itself or whether a single definition can be adequate to explain the entirety of metabolic repertoires.[30]

History of the Duke Lemur Center (DLC) colony

Tracing the history of hibernation science in dwarf lemurs cannot be separated from the history of the colony at the DLC. The first arrival of dwarf lemurs to the DLC was a stroke of serendipity and had nothing to do with hibernation. The DLC (then the Duke University Primate Center [DUPC]) was founded in the 1960s by two biology professors, including one of us (P.H.K.) and John Buettner-Janusch (better known as "BJ"), who moved his primate colony from Yale University to Duke University.[31] Early work spearheaded by BJ was centered around primate evolution, first using primate hemoglobin and later chromosomes as molecular tools for phylogenetic analyses.[32,33] Dwarf lemurs were brought in as part of the menagerie from Madagascar for comparative purposes. Spotty records point to four original founders from the 1960s, with unknown provenance. Three of these (2 F, 1 M) are the genetic founders of today's colony. During the following five decades, the DLC fat-tailed dwarf lemur colony cycled between population booms and bottlenecks,[34] without obvious signs of endogamic depression, and was the source of early scientific inquiries into reproduction, activity, and metabolism.[8–10]

By 2008, when data from the field finally clarified that dwarf lemurs are obligate hibernators, the DLC colony was reduced to a few extraordinarily aged individuals[34] that had never truly hibernated and were no longer genetically or demographically viable. However, serendipity struck again. It turned out that in 1988, several DLC dwarf lemurs were sent to the California Institute of Technology (CIT) where they participated in different aspects of brain research.[35–38] By 2008, eight descendants from the CIT dwarf lemur colony were still alive, albeit with spotty pedigrees. Under the directorship of one of us (A.D.Y.), these second-wave founders were repatriated to the DLC to revitalize the colony and became the source for the next wave of scientific research on primate hibernation.[39,40] So far as we know, this colony is the only breeding population of captive dwarf lemurs.

Hibernation research at the DLC

In the late 2000s, under the leadership of P.H.K., our team attempted to induce hibernation in DLC dwarf lemurs for the first time to study physiological states and particularly sleep patterns. This period of research was characterized by small-scale testing of individual dwarf lemurs with available, but somewhat inadequate, infrastructure.[40] These research attempts demonstrated the potential of dwarf lemurs for studying primate hibernation while highlighting the husbandry and institutional constraints of conducting noninvasive research in a safe manner. Most notably, regulatory agencies in the United States that

govern vertebrate welfare were reluctant to approve protocols that called for food deprivation and subjecting lemurs to cold temperatures below primate standards.

Thus, during the late 2010s, our team designed and implemented a program to facilitate the expression of multiday torpor bout–arousal cycles. This program was rooted in the new understanding of dwarf lemur ecology and based, in part, on our team's experience working with wild dwarf lemurs across Madagascar.[1,2,41] This program included optimizing diets to promote seasonal fattening and fat depletion,[42] subjecting dwarf lemurs to seasonal changes in stable ambient temperature (e.g., 12–15°C), closely monitoring animal activity, body mass, and fat stores, and progressing from seasonal food restriction[43,44] to food deprivation (this study). This approach not only reinforced earlier findings that captive dwarf lemurs can hibernate but incidentally improved reproductive success, prompting a population tripling in just a few years.

With a larger colony and successful induction of seasonal torpor–arousal cycles, we showcase the following two experimental years in which we probed how dwarf lemurs respond to environmental variability during the dry season. In the first year, we asked how food availability influences hibernation expression. We compared torpor–arousal cycles, body mass, and fat stores between food-provisioned and food-deprived individuals under stable cold conditions. In the second year, we asked how temperature variation influences hibernation expression. We tested whether captive dwarf lemurs can continue to thermoconform under fluctuating room temperatures while comparing their temperature profiles, body mass, and fat stores to lemurs under stable cold conditions.

In conjunction with these studies, we explored the utility of biological samples to capture metabolic and physiological processes underpinning hibernation expression.[42,45] In the first year, we examined the gut microbiome between food-provisioned and food-deprived lemurs under stable cold conditions. The gut microbiome, intrinsically intertwined with host metabolism, likely plays a strong role in facilitating hibernation.[46] We specifically tested if food-deprived dwarf lemurs show predictable gut microbiome patterns during hibernation and if eating during this time modulates gut microbial membership and diversity.

Across study years, we measured oxidative stress (8-hydroxy-2′-deoxyguanosine) in urine samples collected from food-deprived and food-provisioned animals under stable cold conditions and/or under fluctuating ones. We added opportunistic samples from animals collected during the non-hibernation season as well. We asked if animals undergoing torpor–arousal cycles from cold stable temperatures experience greater oxidative stress than do active or thermoconforming lemurs under variable temperature conditions.

METHODS

The subjects were 23 dwarf lemurs during the 2021–2022 (n = 14) and 2022–2023 (n = 16) hibernation seasons (Table 1). During the non-hibernation seasons (March–August), the lemurs were maintained singly housed or in social groups on an alternating photoperiod and reverse light cycle under warm conditions (22–25°C) and fed a high-fat diet.[42] In mid-August, they were switched to a high-sugar diet to fatten them ahead of hibernation (see dietary details in Ref. 42; and photoperiod details in Ref. 43). Active and hibernation periods in captivity approximately follow schedules from wild fat-tailed dwarf lemurs in western Madagascar (southern hemisphere), where the core of the hibernation season spans May to the end of October.[47]

FIGURE 1 Photo of the study dwarf lemur "To" inside a nest box, within an enclosure that is in one of the new hibernacula at the Duke Lemur Center.

During the hibernation season, study lemurs were transferred to temperature-controlled hibernacula. In year 1, we used the old hibernacula that consisted of two modified rooms that could be set to different stable temperature profiles. In year 2, we inaugurated and tested new hibernacula that consisted of two environmental chambers with more sophisticated temperature settings, including the ability to fluctuate temperatures. Both hibernacula were furnished with enclosures (dimensions were minimally 61 cm L × 51 cm W × 91 cm H), and each enclosure contained a wooden nest box (25 cm L × 14 cm W × 18 cm H) that mimics the tree holes where this species naturally hibernates (Figure 1). Each animal was assigned to one enclosure, but socially housed animals had access to each other's enclosures and nest boxes. Whenever possible, we kept social groups together. Socially housed dwarf lemurs usually but not always shared nest boxes when hibernating (M.B.B., unpublished data). Fresh water was always freely available.

Prior to each hibernation season, study lemurs were subjected to a biomedical exam, biological sampling, and collaring performed under anesthesia (ketamine; 10 mg/kg, intramuscular). Biological

TABLE 1 Subjects, conditions, demographic and social metadata, and body-mass and tail-girth measurements at the start and end of two hibernation experiments.

							Body mass (g)*		Tail girth (cm)*	
Year	Temperature	Food	Animal	Sex	Age (years)	Social group	Start	End	Start	End
2021–2022	13°C, stable	No	Fr	M	8.2	Singly housed	301	185	7.95	6.25
			Th	M	15.3	Singly housed	243	170	7.25	6.05
			Sa	F	16.3	Group 1	274	182	7.90	6.0
			My	F	1.4	Group 1	313	232	7.50	6.40
			Em	F	6.2	Group 2	286	185	7.20	4.90
			Ko	M	7.2	Group 2	297	176	8.25	5.35
			Do	M	1.2	Group 2	266	180	7.40	5.25
			Cu	M	1.2	Group 2	316	211	8.50	6.50
		Yes	DoC	F	1.3	Singly housed	243	206	7.25	6.25
			Go	M	1.1	Singly housed	301	215	7.25	5.75
			VoC	M	1.3	Singly housed	281	248	7.50	6.75
			WT	M	8.3	Singly housed	296	235	7.75	7.25
			Du	M	6.2	Singly housed	309	211	7.25	6.25
			Au	M	9.3	Singly housed	269	226	7.15	6.35
2022–2023	13°C, stable	No	Fr	M	9.3	Singly housed	244	208	7.50	7.05
			Au	M	10.4	Singly housed	236	188	7.10	6.25
			FrC	F	1.5	Group 3	197	173	6.54	6.15
			Do	M	2.3	Group 3	220	194	7.30	6.95
			SeC	F	2.4	Singly housed	225	185	7.00	6.80
			VoC	M	2.4	Singly housed	220	182	6.80	6.15
	12–30°C, fluctuating	No	To	F	11.4	Group 4	225	203	7.00	6.30
			Me	M	1.5	Group 4	242	212	7.30	6.65
			EB	M	3.5	Group 5	220	174	7.00	5.50
			Al	M	3.5	Group 5	226	185	7.20	6.25
			Bu	M	3.5	Group 5	235	201	7.50	6.70
			DoC	F	2.4	Group 6	226	199	6.75	6.00
			Go	M	2.3	Group 6	224	199	6.60	5.75
			PhC	F	1.5	Group 7	245	213	6.90	6.35
			JaC	F	1.5	Group 7	234	208	6.90	6.25
			WT	M	9.5	Singly housed	196	163	7.20	5.50

*Body-mass and tail-girth values in year 1 from late-October (start) and mid-March (end); in year 2 from December 5 (start) and January 30 (end).

samples included blood for glucose and insulin;[45] oral swabs for telomere analysis;[48] urine for analysis of oxidative stress collected while gently pressing on the bladder and banking at −80°C; and rectal swabs for microbiome analyses collected via inserting a sterile rayon-tipped swab into the base of the rectum, rotating for 10 s, and banking at −80°C. Collaring included outfitting all individuals with an external transmitter with a variable pulse rate that reflected temperature (Advanced Telemetry Systems, M1550, 3.5 g; collar size/body-mass ratio <4%). Transmitters were factory-calibrated in a temperature-controlled chamber, and coefficients from a linear regression were used to associate ppm (pulse per minute) rates with the temperature values. When pressed against the animals' necks, skin temperatures (Tsk) provided a reliable measure of body temperature that we used to estimate torpor expression.[49] We set an external receiver and data logger outside the hibernacula (Advanced Telemetry Systems, R4500), which was programmed to collect and store hourly temperature readings from all collars throughout the study.

Throughout the hibernation season, we weighed lemurs (to the nearest gram) and measured their tail circumferences (in cm) every other week. Whenever possible, we timed weighing events with natural arousals to limit disturbing torpor expression. We used the average of mid-tail and tail-base circumference to estimate tail girth.[44]

Animals were brought to the veterinary clinic at a mid-hibernation timepoint for repeat biological sampling. At the end of the study, they

were brought for a final round of sampling and collar removal, at which point they were returned to the general population for the breeding season and standard feeding protocols were resumed.

Year 1: Study design

In late-October 2021, the 14 study lemurs received exams and collars and were transferred the same day to hibernation chambers. From November 1, 2021 to March 14, 2022, temperatures in both chambers were dropped to stable cold conditions (13°C), selected as a low value but within the range experienced by wild fat-tailed dwarf lemurs in western Madagascar. During this time, eight lemurs housed in one chamber received no food, whereas six lemurs housed in the other chamber continued to receive high-sugar diets daily. All study animals receiving food were singly housed, which prevented any animals eating more than their allotment. Between January 18 and February 2, 2022, lemurs underwent mid-season sampling. On March 14, 2022, we raised temperatures to stable, warm conditions, resumed feeding the lemurs, and removed collars shortly thereafter.

Year 2: Study design

In mid-October 2022, the 16 lemurs were transferred to enclosures in the new hibernation chambers for acclimatization. In early November, they received biomedical exams and collars.

On November 21, 2022, we induced torpor expression in all lemurs by dropping temperatures in both chambers to stable cold conditions (13°C). On this day, all lemurs received their final diet of the year. On December 5, 2022, one chamber with 10 lemurs was transferred to a fluctuating temperature profile designed to approximately mimic the daily temperature profiles of the dry deciduous forests in western Madagascar during the dry season.[16] Specifically, temperatures ranged from 12–30°C over a 24-h period with the coldest temperatures occurring at sunrise (i.e., when white lights were switched on). The second chamber with six lemurs was maintained at stable cold conditions. In late January, lemurs were brought for mid-season exams. Required maintenance of the new chambers necessitated ending this hibernation study early. On January 30, 2023, temperatures in both chambers were artificially raised to standard warm conditions again, we resumed feeding the lemurs each day, and collars were removed shortly thereafter.

Analysis of torpor expression, body mass, and tail girth

For animals under stable cold conditions in both years, torpor expression was assessed for each individual by quantifying the number of hours that Tsk were under 22.5°C, roughly 9°C above ambient temperature. This threshold was set to make sure that we did not overestimate torpor expression, consistent with previous studies.[44] Notably, temperature values from the radio-collars can be affected by ambient temperature when transmitters are not firmly pressed against the lemurs' necks. This was particularly true for those with access to daily food. Visual examination of Tsk of individuals outside of the nest boxes confirmed the temperature threshold clearly differentiated torpid versus active lemurs. For animals under fluctuating temperatures in year 2, we instead calculated the number of hours that the lemurs' Tsk were above 30°C, which was the hottest ambient temperature each day. We use the hours above 30°C as a proxy of active rewarming.

We determined the effect of food availability on torpor expression in year 1 by computing the duration of each torpor bout (in hours). We performed a linear mixed model (LMM) using the *lmerTest* package (version 3.1-3)[50] in RStudio (version 2023.12.0)[51] with R software (version 4.2.1).[52] We entered torpor-bout duration as the dependent variable, food availability (two categories: no food versus daily food) and study day (continuous variable, in days) nested within food availability as the independent variables, and individual lemur as the random term. We considered day 0 as the day the temperatures were dropped. The data were best fitted by a Gaussian distribution following log transformation, as assessed by reduced AIC values. Because this model included many torpor bouts per individual, we computed the average torpor-bout duration for each lemur and used these points in a nonparametric Mann–Whitney test to compare no-food and daily-food groups. We also determined the minimum Tsk for each torpor bout, computed an average for each lemur, and used Mann–Whitney tests to compare patterns between no-food and daily-food groups. We also used Mann–Whitney tests to compare the percentage of body-mass and tail-girth loss between study groups, as well as to compare absolute body mass and tail girth between groups at study onset (i.e., to ensure that values between groups were similar).

For year 2, we compared patterns of body-mass loss to time in torpor for animals under stable cold conditions and time in euthermia for animals under fluctuating conditions. Notably, for animals under stable cold conditions, we calculated the total number of hours spent in torpor and the percentage of mass loss from the day temperatures dropped (November 21, 2022) to the day they increased (January 30, 2023) and compared values using linear regression. For animals under fluctuating conditions, we calculated the total number of hours above 30°C and the percentage of body-mass loss from the day temperatures began fluctuating (December 5, 2022) to the day they stabilized (January 30, 2023) and compared values using linear regression. As in year 1, we also compared patterns of body-mass loss and tail girth between groups using Mann–Whitney tests, although, for this analysis, we used the percentage of mass or girth loss for all animals between December 5, 2022 and January 30, 2023.

Microbiome analyses

To describe gut microbiome profiles during hibernation in captive dwarf lemurs, we used a subset of 14 rectal swabs collected during the mid-season sampling timepoint from animals in year 1 that received no food or daily food. We extracted genomic DNA from swabs using

TABLE 2 Urine samples analyzed for oxidative stress.

Season	Temperature	Food	Number of lemurs	Number of samples	8-OHdG	
					Mean	Range
Hibernation	13°C, stable	No	2	2	333.60	307.52–359.68
		Yes	7	7	178.99	70.42–333.76
	12–30°C, fluctuating	No	7	7	100.15	19.62–152.31
Non-hibernation	22–25°C, stable	Yes	27	69	96.22	16.96–187.36

Abbreviation: 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

commercial kits (DNeasy PowerSoil Pro Kit, Qiagen) and following previous methods.[53] DNA concentrations ranged from 2.9 to 22.0 ng/μL. Extracts were stored at −80°C. Aliquots were shipped to the Argonne National Laboratory (Lemont, Illinois, USA) for library preparation and amplicon sequencing. We targeted the V4 region of the 16S rRNA gene using previous methods.[53]

We processed raw reads using a QIIME 2 pipeline (version 2022.8).[54] Samples were represented by 41,085–98,835 high-quality, raw sequence reads. We binned sequences in amplicon sequence variants (ASVs) based on 100% identity, removed ASVs present in one sample, assigned taxonomy using the SILVA 138 database,[55] removed mitochondrial and chloroplast sequences, and collapsed ASVs at genus-level resolution. We computed alpha diversity, namely, observed features and Faith's phylogenetic diversity, rarefying to a depth of 10,000 reads/samples at the time of metric computation.

We compared microbiome features in samples from dwarf lemurs that received no food versus daily food during hibernation. We used nonparametric Mann–Whitney tests to compare observed features and phylogenetic diversity. We used MaAsLin2 (version 1.12.0) to compare the relative abundance of microbial genera.[56]

Oxidative stress analysis

Over multiple years of study, we amassed a set of urine samples for analysis of oxidative stress (Table 2). We transferred samples to Eurofins SF Analytical DBA Craft Technologies for assays of 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Colorimetric/Competition ELISA kit ab201734, intra- and inter-assay CV% <9%). This is a widely used biomarker of oxidative DNA damage that has been validated and used across several mammal and primate species, including lemurs, monkeys, apes, and humans.[57–61] Unfortunately, we were not able to correct for urine concentration by running a separate creatinine assay. Thus, results should be taken with caution. We compared 8-OHdG in urine samples (50 μL per sample independently assayed) from dwarf lemurs hibernating under different conditions and from those sampled during the non-hibernation seasons. We ran an LMM using 8-OHdG as the dependent variable, season (four categories: cold no food; cold daily food; fluctuating no food; non-hibernation season) as the explanatory variable, and individual lemur as the random term. Log transformation significantly improved model fit.

RESULTS

Torpor expression

As expected, dwarf lemurs in year 1 maintained under cold conditions without food during the hibernation season generally alternated between multiday torpor bouts punctuated by periodic arousals. In contrast, dwarf lemurs maintained under cold conditions with daily food expressed temperature profiles more consistent with daily torpor; they showed torpor bouts of under 24 h and aroused each day to eat. The difference in torpor bout duration was significant between study groups (LMM: $t_{19.87} = 2.477$, $p = 0.022$; Figure 2A). The longest single torpor bout for the no-food group was 267 h (11.1 days), and all individuals had one torpor bout of a minimum of 200 h (8.3 days). In contrast, the longest torpor bout for the daily-food group was just 18 h (0.75 days). Torpor bouts for the no-food group were 53.8 ± 9.3 (mean ± SD) hours (2.2 ±0.4 days) and for the daily-food group were 6.7 ± 0.4 h (0.28 ± 0.02 days). This difference was significant ($U = 0$, $p < 0.001$; Figure 2B). Interestingly, torpor bouts got significantly longer as the study progressed; however, this pattern was much stronger for the no-food group (LMM: $t_{1031} = 20.283$, $p < 0.0001$) than for the daily-food group (LMM: $t_{1033} = 2.048$, $p = 0.04$). As the study was concluded after 4.5 months, however, it is likely that we did not capture patterns representing the entirety of the hibernation season as torpor bouts shorten prior to emergence from hibernation.[28]

The minimum Tsk achieved during torpor bouts was also lower in the no-food group compared to the daily-food group ($U = 0$, $p < 0.001$; Figure 2C). Although minimum Tsk during torpor for the no-food group was 14.8 ± 1.3°C (mean ± SD), it was 17.0 ± 0.4°C for the daily-food group. At the start of the experiments, individuals from the no-food and daily-food groups had comparable body mass (mean ± SD; 287 g ± 25 and 283 g ± 24, respectively; $U = 21$, $p = 0.731$) and tail girth (7.7 cm ± 0.5 and 7.4 cm ± 0.2, respectively; $U = 12$, $p = 0.128$) values. Both study groups' individuals lost body mass and tail girth across the hibernation season, but the patterns were attenuated by food availability. Unsurprisingly, dwarf lemurs with access to food lost a significantly smaller percentage of body mass ($U = 3$, $p = 0.005$; Figure 2D) and tail girth ($U = 2$, $p = 0.002$; Figure 2E) compared to lemurs with access to no food each day.

As expected, dwarf lemurs maintained under stable cold conditions in year 2 without food during the hibernation season alternated between torpor bouts punctuated by periodic arousals (for an example

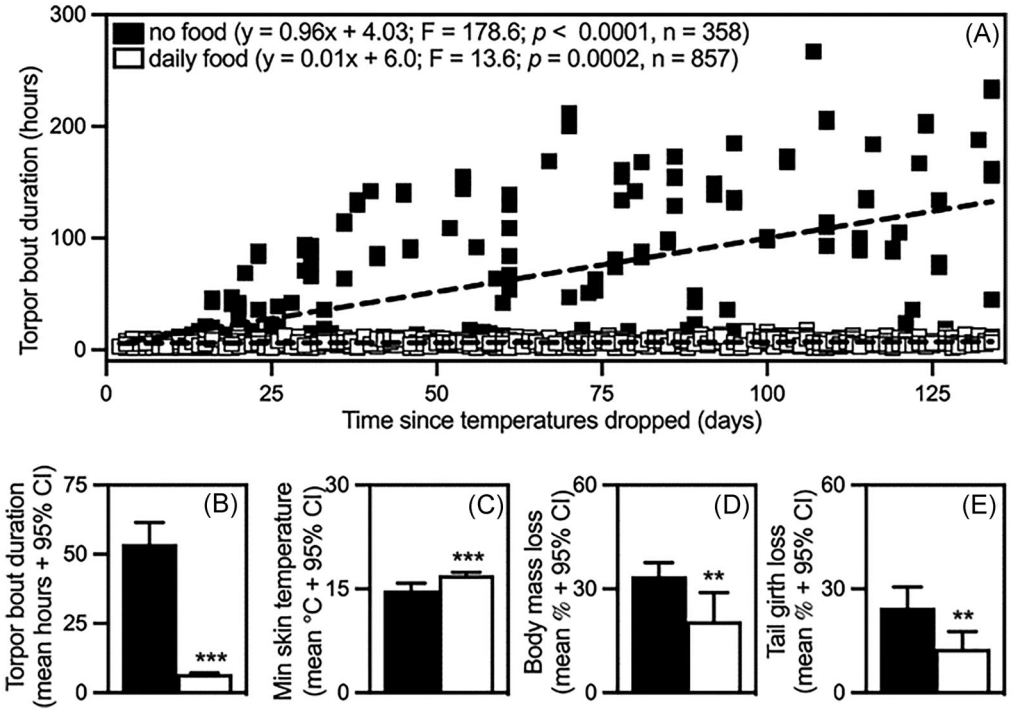

FIGURE 2 Patterns of torpor expression, body-mass and tail-girth loss in year 1 relative to food availability during the hibernation season. Depicted here, for food-deprived (black) and food-provisioned (white) dwarf lemurs, are the (A) durations of each individual torpor bout relative to the days since temperatures dropped to 13°C, with equations and statistics for regression lines, (B) average torpor bout duration, (C) average minimum skin temperature measured during each torpor bout, and percentage of (D) body-mass and (E) tail-girth loss across the hibernation season. $^{**}p < 0.01$; $^{***}p < 0.001$.

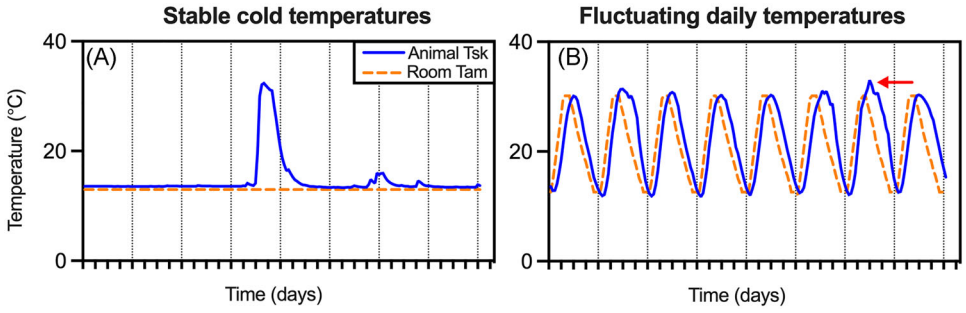

FIGURE 3 Patterns of torpor expression in year 2 relative to temperature profiles during the hibernation season. Shown here are the skin temperature (Tsk) (solid blue line) and ambient temperature (Tam) (dotted orange line) profiles for dwarf lemur (A) animal "FrC" under stable cold temperatures and (B) animal "DoC" under daily fluctuating temperatures across 8 days. Vertical dotted lines denote 24-h periods and ticks along the *x*-axis are spaced every 6 h. The red arrow points to the case in (B) where Tsk rose above 30°C.

of an arousal between torpor bouts, as captured by Tsk data, please see Figure 3A). The longest single torpor bout was 208 h (8.7 days), and torpor bouts were (mean ± SD) 50 ± 12.7 h (2.1 ± 0.5 days). All individuals experienced one torpor bout of a minimum of 113 h (4.7 days).

Dwarf lemurs maintained under fluctuating temperature conditions without food passively tracked increasing and decreasing ambient temperatures, sometimes displaying Tsk above 30°C (i.e., active rewarming) during the hot phase (Figure 3B). Some lemurs actively rewarmed briefly every day, whereas others spent up to 30% of the study days without showing arousals. Lemurs spent, on average, 14% of the days without showing active rewarming. Daily Tsk periods above 30°C were 4.9 h ± 1.4 (mean ± SD) across all individuals. During daily check-ups, dwarf lemurs were often observed outside their nest boxes during the hot phase but were always observed inside the boxes during the cold phase. These movements may coincide with periods of active rewarming shown in the Tsk profiles.

Patterns of torpor expression correlated with patterns of body-mass loss for lemurs under both temperature regimens. For lemurs under stable cold conditions, the percentage of body-mass loss from the day the temperatures dropped on November 21, 2022 to the day

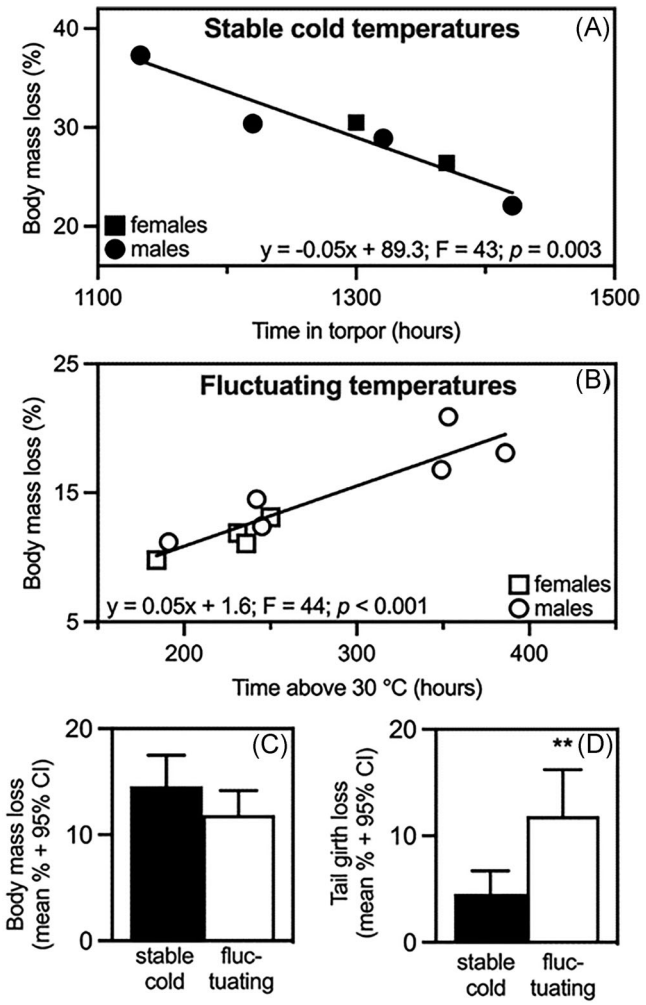

FIGURE 4 Patterns of body-mass and tail-girth loss in year 2 relative to temperature profiles during the hibernation season. Depicted here are the percentages of body-mass loss for dwarf lemurs hibernating under (A) stable cold temperatures relative to total time spent in torpor and (B) fluctuating daily temperatures relative to total time spent above 30°C for females (squares) and males (circles). Equations and statistics for regression lines are provided. Also shown are the average percentages of (C) body-mass and (D) tail-girth loss. Note that (A) depicts patterns from November 21 to January 30, whereas (B–D) depict patterns from December 5 to January 30. $^{**}p < 0.01$.

they were raised on January 30, 2023 negatively correlated to the total number of hours in torpor during the same time period ($F_{1,4} = 43.34$, $R^2 = 0.92$; $p = 0.003$; Figure 4A). For lemurs under fluctuating conditions, the percentage of body-mass loss from the day temperatures began fluctuating on December 5, 2022 to the day they stabilized on January 30, 2023 positively correlated to the total number of hours spent above 30°C during the same time period ($F_{1,8} = 43.99$, $R^2 = 0.86$; $p < 0.001$; Figure 4B). Curiously, dwarf lemurs under fluctuating conditions lost a similar percentage of body mass across the hibernation season compared to those under stable cold conditions ($U = 14$, $p = 0.09$; Figure 4C) when scaled to the same time period (December 5, 2022–January 30, 2023); however, they lost significantly more girth in their tails ($U = 4$, $p = 0.003$; Figure 4D).

The gut microbiome

Dwarf lemurs maintained under cold conditions during the hibernation season had markedly different gut microbiomes if they received no food versus daily food (Figure 5). Both microbiome richness (Figure 5A) and phylogenetic breadth (Figure 5B) were greater in the fed groups compared to the unfed group ($U = 0$, $p < 0.001$ for both comparisons).

Overall, we identified 144 microbial taxa present in at least 1 sample, and 63 of these were differently enriched between study groups. Animals in the no-food group had consortia dominated by *Bifidobacterium* (8.4%), *Bacteroides* (25.1%), Aerococcaceae (15.1%), and *Morganella* (18.6%) that together accounted for 67% of the gut microbiome. In contrast, animals in the daily-food group had consortia dominated by *Campylobacter* (33.4%), *Mycoplasma* (18.1%), and *Bacteroides* (5.4%) that together accounted for 56.9% of the gut microbiome. All these taxa were significantly different between groups ($p < 0.001$ for all comparisons). For full results of differential abundance testing, see the Supporting Information section.

Oxidative stress

We found significant variation in concentrations of urinary 8-OHdG relative to condition ($F_{77.71} = 8.06$, $p < 0.001$; Figure 6). Outside of the hibernation season, dwarf lemurs had reduced concentrations of 8-OHdG compared to those during the hibernation season housed under cold conditions without ($t_{80.10} = -3.760$, $p < 0.001$) and with ($t_{74.56} = 3.275$, $p = 0.002$) food. Curiously, outside of the hibernation season, dwarf lemurs had similar concentrations of 8-OHdG compared to those during the hibernation season housed under fluctuating temperatures without food ($t_{78.35} = 0.083$, $p = 0.934$).

DISCUSSION

Through noninvasive experimental methods, we demonstrated that captive-born dwarf lemurs not only maintain the physiological capacity to undergo torpor–arousal cycles under stable cold temperatures but also continue to thermoconform under fluctuating ones. Although these two hibernation styles may be energetically equivalent,[16,17] and animals in our study did lose similar body mass, torpor–arousal cycles and passive rewarming are not necessarily physiologically equivalent. Notably, lemurs thermoconforming under variable temperature conditions lost a greater percentage of tail girth but showed lower levels of oxidative stress compared to those undergoing torpor–arousal cycles under stable cold conditions. These differences raise curious questions about the timing and rates of lipid metabolism linked to environmental heterogeneity. That both captive and wild dwarf lemurs can continue to thermoconform (i.e., passively track fluctuating daily temperatures

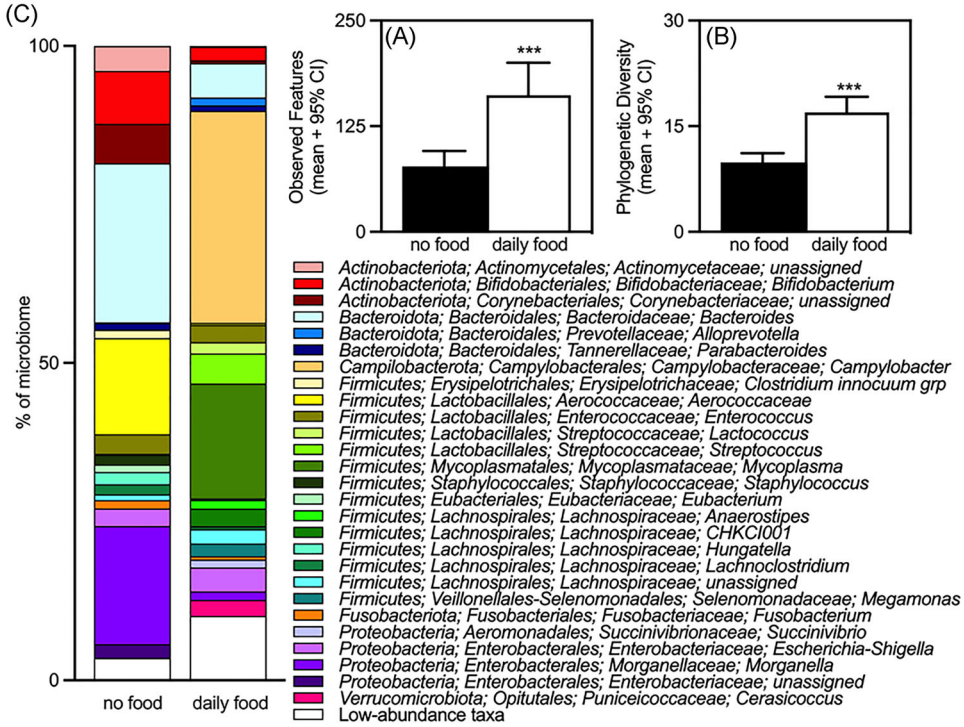

FIGURE 5 Gut microbiome features of dwarf lemurs maintained at stable cold temperatures during the hibernation season without and with daily food provisioning. Depicted here are metrics of alpha diversity, including (A) microbiome richness and (B) phylogenetic diversity for unfed (black) and fed (white) lemurs. Microbiome composition is depicted as (C) stacked bar charts and highlights taxa that accounted for >1% of the microbiome across conspecifics within conditions. Color key of dominant microbial genera is provided in-text, and white refers to the summation of all taxa that failed to reach the 1% cutoff. ***$p < 0.001$.

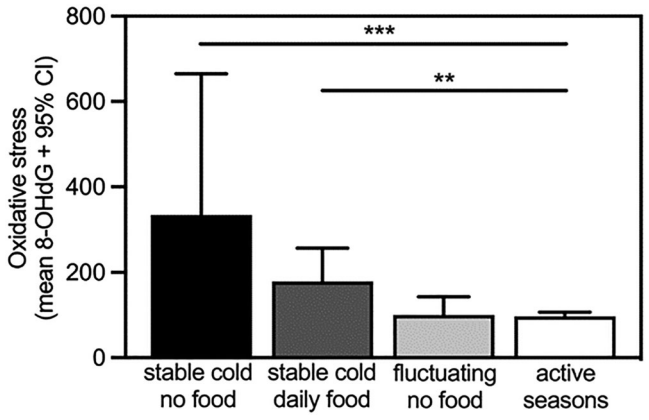

FIGURE 6 Patterns of oxidative stress, as captured by urinary 8-OHdG in dwarf lemurs housed under stable-cold conditions without (black) and with (dark gray) food and under fluctuating temperature profiles without food (light gray) during the hibernation season, as well as during the active seasons (white). **$p < 0.01$; ***$p < 0.001$.

without arousing) is in stark contrast to many temperate hibernators for whom increasing temperatures would terminate hibernation, [29,62] though there are exceptions to these patterns.[63] Our results ultimately underscore the flexibility inherent to survival in the tropics given the diversity of climates and conditions, including warm and fluctuating temperatures even during the coldest times of the year.[23,29]

This flexibility is further highlighted by our experiment of food provisioning. Dwarf lemurs under stable cold conditions during the dry season with food available surprisingly continued to feed daily, despite having copious fat stores. In the wild, dwarf lemurs do not eat or drink for up to 7 months during the dry season when food and water are scarce. In addition to energy and water conservation, hibernation may provide protection from predators, as it had been suggested for other hibernators.[64,65] In this context, daily feeding, despite fat storage, may be a result of captivity and maladaptation; another explanation may relate to the torpor optimization hypothesis.[66] This hypothesis posits that hibernation should be expressed according to energy availability. When under energy surplus, hibernation is limited to avoid the associated physiological costs of arousals. In line with this hypothesis, the food-provisioned dwarf lemurs expressed shorter and shallower torpor bouts. This potentially served to limit the degree of thermogenesis these lemurs needed to fully arouse, as evidenced by their intermediate levels of oxidative stress. That they used torpor at all may point to insufficient calories required to stay euthermic throughout the season, as food was not available ad libitum. That they continue to eat at all, rather than commit fully to hibernation, may point to a degree of hibernation plasticity in this system and echoes patterns seen in a temperate hibernator, the garden dormouse.[67]

As tropical and flexible obligate primate hibernators, dwarf lemurs thus emerge as an alternate system for the study of metabolism, with clear relevance for human health.[45] For starters, dwarf lemurs can

reduce metabolism despite warm ambient temperatures[17]; leading to the potential for inducing metabolic depression in humans without relying on hypothermic conditions.[68] Moreover, such metabolic flexibility is manifested in other physiological processes, such as the dwarf lemurs' ability to sleep while passively rewarming[40] and their capacity to adjust heart rates and breathing patterns relative to metabolic activity.[69] Dwarf lemurs may even prove useful for understanding neurological processes associated with human aging as they can accumulate iron deposits and amyloid burden that are perhaps remnants of protective mechanisms employed by the brain to cope with torpor.[70] Further mining of their genomes and transcriptomes could help determine the molecular underpinnings of metabolic and physiological flexibility,[39,71] with applications for gene therapies.

Beyond hibernation itself, how dwarf lemurs prepare to hibernate has additional relevance. Unlike many temperate hibernators that rely on dietary fats to fatten,[72] dwarf lemurs naturally forage on ripe fruits and seasonally fatten by endogenously converting sugars to lipids.[18,73] Remarkably, they do so without showing signs of glucose intolerance.[45] This particular method of fattening has potential value for research not only of metabolic syndromes, but also for studies of the gut microbiome. In Madagascar, dwarf lemurs show seasonal reconfigurations of the gut microbiome associated with sugary diets and the hibernation cycle.[53] Despite captivity often "westernizing" or disrupting species-specific gut consortia,[74] many of the dominant microbes we identified in DLC lemurs matched those of their wild congeners, underscoring that captive lemurs may be relevant proxies for microbiome science. Unfed lemurs undergoing torpor–arousal cycles in Madagascar and at the DLC showed blooms of both the Aerococcaceae family and the Actinobacteriota phylum, consistent patterns that beg explanation. In contrast, lemurs feeding on high-sugar diets, regardless of season, showed abundant *Mycoplasma*, a taxon historically clustered in the Mollicutes class that is more easily linked to sugary diets and obesity in humans and model systems.[75,76] Whether these microbes serve essential functions for their hosts that enable hibernation or merely respond to differences in the gut environment remains to be tested. Taken together, however, these clues point to both endogenous and microbial mechanisms—from insulin receptors, adipose signaling, and hormones to fermentation and nutrient recycling—that are needed to counteract seasonal sugar intake and the feast–fast cycle without ill health effects.

Future directions

Does primate hibernation extends primate longevity (i.e., do dwarf lemurs that hibernate more live longer?). On the one hand, from the CIT dwarf lemurs, we know that those unable to hibernate and fed poor diets ended up riddled with tumors.[38] On the other hand, from the DLC dwarf lemurs, we know that those unable to hibernate but kept under more appropriate conditions can live extraordinarily long lives for animals of ~200 g.[34] Indeed, the dwarf lemur "Jonas" famously lived at the DLC for almost 30 years.[77] At the other end of the spectrum, cumulative torpor–arousal cycles, particularly from cold temperatures, impose a physiological cost,[78] as evidenced by our pilot analysis of oxidative stress. Under the torpor-optimization hypothesis, animals try to avoid this scenario when energetic demands can be met otherwise.[66] This perhaps leads to the assumption that coping with extreme physiological stress may limit longevity. At the same time, hibernators have mechanisms to offset these physiological costs, and dwarf lemurs can seemingly repair and even elongate telomeres while hibernating.[48] These hints, while tantalizing, cannot provide a clear answer to the longevity question.

With a more robust colony and new hibernation infrastructure, the DLC could be poised to implement a series of decades-long experiments and address fundamental questions about hibernation, flexibility, and longevity in primates. For example, animals individually known and tracked from birth could be safely placed into lifelong conditions of minimal seasonality, seasonal torpor-arousal cycles, and/or passive rewarming. Such long-term experiments could determine the environmental conditions that reduce physiological costs and extend longevity, with clear implications for human biomedicine. In conjunction with such experiments, researchers could also probe patterns of weight gain and loss linked to resource availability and metabolism to clarify physiological underpinnings of fertility and reproduction in both sexes. In dwarf lemurs, the reproductive season directly follows the hibernation season. We currently lack insight into how the hormonal cascades that underlie reproduction in either sex relate to the metabolic processes experienced just after hibernation, although this is a rich area for follow-up research that might shed light on primate reproductive biology more generally.

The value of such long-term experiments could extend beyond relevance to human health, in line with the DLC's historic mission of conservation science.[79] Such experiments could model the stochastic and hypervariable environments of Madagascar,[80] and would probe how dwarf lemurs respond to diverse ecological challenges pertinent to understanding their potential resilience to ongoing habitat and climate change.[1] Ultimately, research on captive dwarf lemurs could inform conservation action through targeted reforestation efforts (i.e., planting species with phenological breadth and nutritional value) or species/habitat compatibility for potential translocation efforts. Lemurs are, after all, among the most endangered vertebrates on Earth.[81] In keeping with the DLC's mission of non-harmful science, safe animal experimentation could be coupled with strategic sampling, a push for emerging technologies that provide measurements of living animals, the development of cell lines that can be exposed to a greater range of laboratory conditions, and deep sequencing of lemur and microbial genomes and transcriptomes analyzed via robust computational biology.

Although the origin of the DLC dwarf lemur colony, over half a century ago, was serendipitous and remains somewhat murky, the founders' descendants have persisted through periods of notable uncertainty, even managing to be involved in sporadic research projects along the way. Over the last 25 years, extensive fieldwork on the ecophysiology of hibernation has demonstrated the dwarf lemurs' unique physiological traits, and a new generation of DLC dwarf lemurs became the focus of a new research program, holding promise for

both their wild kin and human relatives. To promote such promises, a new consensus should emerge that combines efforts among scientists united by an interest in hibernation, from those in biomedicine who apply new technologies, ingenuity, and experimental rigor to human health, to those in biology, ecology, and primatology who can investigate evolutionary questions that address current conservation challenges.

AUTHOR CONTRIBUTIONS

M.B.B., P.H.K., and A.D.Y. conceived of the DLC hibernation initiative with support from E.E.E. M.B.B and L.K.G. designed the study components contained within this manuscript. M.B.B., L.K.G., and K.H.W. were responsible for study implementation and data collection with support from E.E.E. M.B.B. and L.K.G performed laboratory work, bioinformatics, and statistical analyses and wrote the manuscript. All authors approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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