FEMS Microbiology Ecology, 2022, 98, 1–10
DOI: 10.1093/femsec/fiac070
Advance access publication date: 9 June 2022
Research Article

Variation in gut microbiome structure across the annual hibernation cycle in a wild primate

Lydia K. Greene[1,2,*], Jean-Basile Andriambeloson[3], Hoby A. Rasoanaivo[4], Anne D. Yoder[2], Marina B. Blanco[1,2]

[1]The Duke Lemur Center, 3705 Erwin Road, Durham, NC 27705, United States
[2]Department of Biology, Duke University, Durham, NC 27708, United States
[3]Department of Zoology and Animal Biodiversity, Faculty of Science, University of Antananarivo, Antananarivo, Madagascar
[4]Department of Science and Veterinary Medicine, Faculty of Medicine, University of Antananarivo, Antananarivo, Madagascar
*Corresponding author: The Duke Lemur Center, 3705 Erwin Road, Durham, NC 27705, USA. E-mail: lydiakgreene@gmail.com
One sentence summary: Wild dwarf lemurs in Madagascar show seasonal reconfigurations of the gut microbiome across the annual hibernation cycle, with microbial diversity tracking the host's reliance on dietary fruit sugars.
Editor: Cindy Nakatsu

Abstract

The gut microbiome can mediate host metabolism, including facilitating energy-saving strategies like hibernation. The dwarf lemurs of Madagascar (*Cheirogaleus* spp.) are the only obligate hibernators among primates. They also hibernate in the subtropics, and unlike temperate hibernators, fatten by converting fruit sugars to lipid deposits, torpor at relatively warm temperatures, and forage for a generalized diet after emergence. Despite these ecological differences, we might expect hibernation to shape the gut microbiome in similar ways across mammals. We, therefore, compare gut microbiome profiles, determined by amplicon sequencing of rectal swabs, in wild furry-eared dwarf lemurs (*C. crossleyi*) during fattening, hibernation, and after emergence. The dwarf lemurs exhibited reduced gut microbial diversity during fattening, intermediate diversity and increased community homogenization during hibernation, and greatest diversity after emergence. The *Mycoplasma* genus was enriched during fattening, whereas the *Aerococcaceae* and *Actinomycetaceae* families, and not *Akkermansia*, bloomed during hibernation. As expected, the dwarf lemurs showed seasonal reconfigurations of the gut microbiome; however, the patterns of microbial diversity diverged from temperate hibernators, and better resembled the shifts associated with dietary fruits and sugars in primates and model organisms. Our results thus highlight the potential for dwarf lemurs to probe microbiome-mediated metabolism in primates under contrasting conditions.

Keywords: gut microbiota, *Cheirogaleus*, torpor, lemur, Madagascar

Introduction

Hibernation, i.e. controlled metabolic depression used to conserve energy, is present in members of all major mammalian lineages, but is rare among primates (Blanco *et al.* 2018). The dwarf lemurs of Madagascar (*Cheirogaleus* spp.) are the only obligate primate hibernator and have, therefore, emerged as fascinating models for ecological studies of metabolism with relevance to human biomedicine (Blanco *et al.* 2018). Dwarf lemurs hibernate for 4–7 months per year, and unlike temperate hibernators, can do so under cold, warm, or thermally unstable conditions in subtropical forests (Dausmann 2014, Blanco *et al.* 2018). While hibernating, dwarf lemurs rely on significant fat depots to fuel metabolism (Fietz *et al.* 2003, Blanco *et al.* 2018). Unusually, these lipid reserves are deposited in the preceding "fattening" season predominately via endogenous conversion of fruit sugars (Fietz and Ganzhorn 1999). At the extreme, dwarf lemurs can double their body mass in anticipation of hibernation (Fietz and Ganzhorn 1999, Fietz and Dausmann 2006). While such weight gain in humans is associated with notable health concerns (Mokdad *et al.* 2003), this seasonal cycle of feasting and fasting, underpinned by switches between sugar and lipid metabolism, is natural for these primates. Decades of research on hibernating mammals, including more recent work on dwarf lemurs, have asked how animal physiology copes with seasonal metabolic depression and weight gain (Geiser and Ruf 1995, Carey *et al.* 2003, Dausmann *et al.* 2005, 2009, Dausmann and Blanco 2016); however, we are only now beginning to understand the associated consequences to the microbial communities, especially in the gut, that must also withstand these contrasting conditions.

Gut microbiome data on dwarf lemurs are scant, but evidence from other hibernating mammals (e.g. bears, squirrels, and bats) has shown that gut microbiomes generally differ between active and hibernation seasons (Sommer *et al.* 2016, Carey and Assadi-Porter 2017, Xiao *et al.* 2019). During the active season, community diversity and density tend to be relatively high (Stevenson *et al.* 2014, Sommer *et al.* 2016, Xiao *et al.* 2019). Consortia are dominated by taxa that thrive on food-derived substrates, especially members of the *Firmicutes* phylum that specialize on plant polysaccharides (Carey and Assadi-Porter 2017). These *Firmicutes*-dominant consortia may play a specific role in the preparation for hibernation. In yellow-bellied marmots (*Marmota flaviventer*), *Firmicutes* are positively correlated to rates of weight gain (Johnson 2021), and germ-free mice colonized with gut microbes from active brown bears (*Ursus arctos*) gain more weight and deposit more fat than do those colonized with consortia from hibernating bears (Sommer *et al.* 2016).

In contrast to the active seasons, the lack of food during hibernation changes the conditions and available nutrients in the digestive tract and is associated with seasonal reconfigurations of the gut microbiome in squirrels and bears: community di-

Received: February 23, 2022. **Revised:** May 7, 2022. **Accepted:** June 6, 2022

versity and density generally decrease, and microbes, like *Akkermansia* and many *Bacteroidetes*, that can survive on host-derived substrates dominate the gut (Sommer *et al.* 2016, Carey and Assadi-Porter 2017). The specific role of fasting is underscored by data from species that rely on food caches instead of fat reserves during hibernation. Syrian hamsters (*Mesocricetus auratus*), showed minimal seasonal reconfigurations of the gut microbiome under conditions of food provisioning; under fasted conditions (for several days), the hamsters showed larger seasonal changes in microbiome composition (Sonoyama *et al.* 2009). During arousals, when hibernators briefly rewarm and reactivate metabolism between prolonged bouts of metabolic depression, microbiome membership changes in horseshoe bats (*Rhinolophus ferrumequinum*) (Xiao *et al.* 2019) highlighting periodic spurts in microbial activity throughout the hibernation season. This activity is rooted in the metabolism of mucin, glycoproteins, and sloughed epithelial cells, and the synthesis of by-product short-chain fatty acids (Carey and Assadi-Porter 2017). During arousals, short-chain fatty acid concentrations increase in Arctic ground squirrels (*Urocitellus parryii*; Stevenson *et al.* 2014): These acids are likely key substrates and nutrients for hosts that otherwise rely predominantly on lipid oxidation to fuel arousals.

Dwarf lemurs have the potential to improve our understanding of hibernation–microbiome dynamics. Specifically, they provide opportunity to ask if hibernation shapes the microbiome across phylogenetically and ecologically divergent hosts in similar or species-specific ways. Most mammalian guts broadly harbor similar microbial memberships at phylum, class, and even family level resolution (Nishida and Ochman 2018, Rojas *et al.* 2021). Lower-level taxonomic resolution, and taxon abundance, are shaped more by host evolutionary history and current environmental conditions (Ley *et al.* 2008, Greene *et al.* 2019, Youngblut *et al.* 2019). Thus, we might expect dwarf lemurs to exhibit similar reconfigurations in the abundances of the *Firmicutes* and *Bacteroidetes* phyla, or in diversity metrics, across the hibernation cycle relative to other hosts (Sommer *et al.* 2016, Carey and Assadi-Porter 2017). Nevertheless, the microbial genera and species underlying such patterns are perhaps more likely to be shared with other primate and lemur lineages. If true, such results would yield insight into how primate-associated microbes survive or flourish under extreme host conditions, including feasting and fasting, and heating and cooling.

Relative to many other hibernators, dwarf lemurs also differ in the preparation for and expression of hibernation, allowing for comparisons of microbiome dynamics across conditions. Unlike most hibernating rodents that fatten in the active season by depositing dietary lipids (Hill and Florant 1999, Munro and Thomas 2004), dwarf lemurs fatten primarily by endogenously converting fruit sugars to lipids (Fietz and Ganzhorn 1999, Blanco *et al.* 2022), especially oleic acid (Fietz *et al.* 2003). Diets high in sugars vs. fats establish different nutrients and conditions within the gut environment and can differentially modulate microbiome diversity, composition, and function (Singh *et al.* 2017, Dahl *et al.* 2020). Sugar vs. fat-based diets also change the composition of fatty acids available to hosts (and potentially to microbes) during the hibernation season (Blanco *et al.* 2022). Unlike most bears that hibernate at near-euthermic temperatures (Tøien *et al.* 2011) and Arctic rodents that hibernate at near-freezing temperatures (Boyer and Barnes 1999, Barnes and Buck 2000), dwarf lemurs can hibernate under variable and strongly fluctuating temperatures (Blanco *et al.* 2018). These temperature ranges may select for different microbial members and functions that can variably withstand warm, cool, or unstable conditions (Sepulveda and Moeller 2020, Huus and Ley 2021), and thereby differentially contribute or respond to host physiology during hibernation.

Here, we provide the first comparison of gut microbiome structure in wild dwarf lemurs across the annual hibernation cycle. We focus on the population of furry-eared dwarf lemurs (*Cheirogaleus crossleyi*) inhabiting the Tsinjoarivo Forest, a high-altitude rainforest in eastern Madagascar (Fig. 1). At this site, dwarf lemurs fatten at the end of the rainy season, hibernate for 4–5 months during the dry season (Blanco *et al.* 2013), and emerge to forage primarily on flowers, insects, and unripe fruits (MBB, unpublished data). While hibernating, dwarf lemurs at Tsinjoarivo experience thermally stable conditions in underground burrows (~15°C), where they undergo multiday bouts of metabolic depression (i.e. torpor) punctuated by short periods of euthermia (i.e. arousals). Using a capture–collar–release–recapture design and nonharmful sampling methods, we determine the diversity, variability, and membership of the dwarf lemur gut microbiome at three time points—during the fattening season, hibernation season, and after emergence from hibernation. Under the hypothesis that seasonal variation in available nutrients linked to the feast-fast cycle underlies gut microbiome structure, we expect the hibernation season, relative to the active seasons, to be associated with depleted gut microbiome diversity and reduced variability among individuals, a reduction of *Firmicutes* and other taxa known to specialize on plant fibers, but an increase in *Bacteroidetes* and *Akkermansia*. We also expect the dwarf lemurs to harbor distinct gut microbiomes during the fattening season and after emergence from hibernation, linked to the differential consumption of high-sugar vs. generalized diets.

Materials and methods

Study site and subjects

We set camp at Andasivodihazo (S19.6801 E47.7707), a forest fragment on the western side of the Tsinjoarivo Forest in 2019 during the fattening season (February), midhibernation season (July), and after emergence from hibernation (October). In the fattening season, we captured eight dwarf lemurs using established methods (Blanco and Rahalinarivo 2010, Blanco *et al.* 2013; Table 1). These lemurs were brought to camp for microbiome sampling (see below) and outfitted with external collars carrying radio transmitters. These radio-transmitters possessed archival tags that record and store temperature every 60 minutes (ARC 400, 10 g, Advanced Telemetry Systems, Isanti, MN; collar size/body mass ratio of < 4%). Hourly temperatures provide an estimate of skin temperature, which in turn, provides a reliable proxy for time spent in torpor (Blanco *et al.* 2013). We released collared lemurs at their initial capture location at sunset the same day. One animal "Mh" was lost in the following days to predation.

In the hibernation season, we used telemetry to track study lemurs to their underground hibernacula. We unearthed individuals, brought them to camp for sampling and recollaring, and returned them to their hibernacula that afternoon, following established methods (Blanco *et al.* 2013). We did not recollar juvenile "Ih" who would become too light to support a collar after emergence. After the hibernation season ended, we tracked lemurs to their tree-hole sleeping sites and placed baited traps nearby. We recaptured four individuals and caught three new individuals. Captured lemurs were brought to camp for sampling and collar removal and were released at their sleeping site that afternoon. One individual "Mb" was subsequently recaptured for collar removal by the field team after the sampling season ended and one

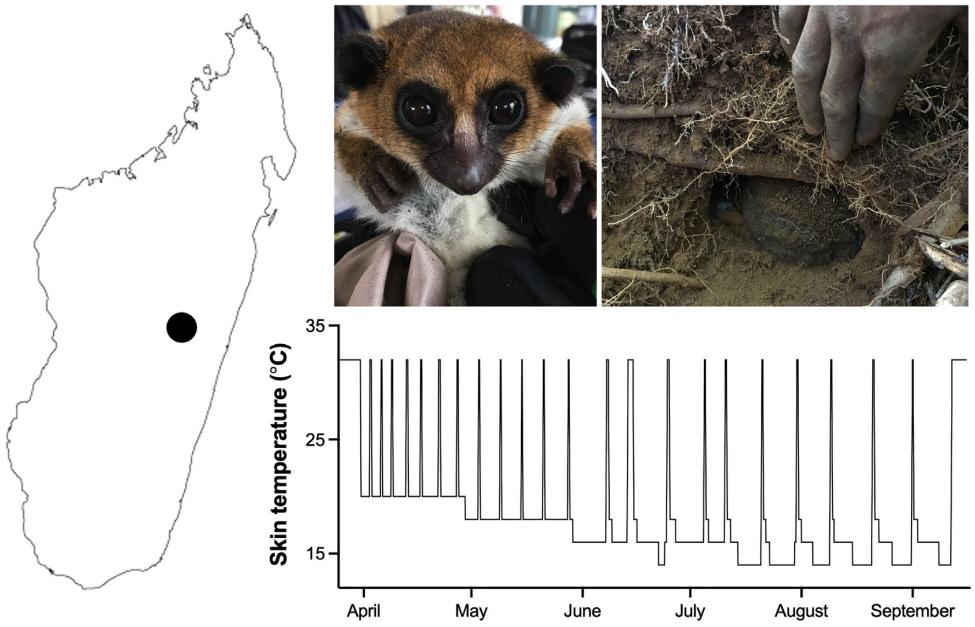

Figure 1. Map of Madagascar highlighting the location of the Tsinjoarivo Forest; photos of furry-eared dwarf lemurs "Mb" during sampling and "Ma" in his underground hibernaculum; and a schematic representation of the skin temperature profile for dwarf lemur "Ra" during the hibernation season from April through September. This schematic depicts skin temperature data collected by the lemur's collar throughout the hibernation season. Each peak corresponds to an arousal, when the lemur briefly rewarmed before depressing metabolism for a subsequent torpor bout. Torpor bouts occurred at lower temperatures as the hibernation season progressed, due to decreasing ambient temperatures of the underground hibernaculum in the core of the dry season.

Table 1. Study subjects and sampling.

			Sampled:		
Individual	**Sex**	**Age**	Fattening	Hibernation	After emergence
Faniry (Fa)	F	Adult	x	x	x
Mahery (Ma)	M	Adult	x	x	x
Razafy (Ra)	F	Adult	x	x	x
Santatra (Sa)	M	Adult	x	x	x*
Narcisse (Na)	M	Subadult	x	x	
Mbola (Mb)	M	Adult	x	x	
Ihary (Ih)	M	Juvenile	x	x	
Malahelo (Mh)	M	Subadult	x		
Tsiory (Ts)	F	Adult			x
Bera (Be)	M	Adult			x
Onja (On)	F	Adult			x

*Sample failed to sequence well and was subsequently dropped from analyses.

individual "Na" was lost shortly after emergence due to predation. Our total sample sizes are eight lemurs in the fattening season; seven in the hibernation season; and seven after emergence from hibernation.

Samples, sample preparation, and sequencing

Given the challenge of securing fecal samples from torpid animals, we collected samples for microbiome profiling across seasons using rectal swabs. During animal captures, we inserted a sterile, rayon-tipped swab into the base of the rectum and gently rotated the swab for 15 seconds. Swab tips were snipped into sterile tubes with scissors prewiped with ethanol and submerged in microbiome buffer (OMNIgene.GUT, DNA Genotek; Brown *et al.* 2018). This buffer has previously been shown to accurately preserve lemur gut microbiomes (Greene *et al.* 2021a). Samples were kept at ambient temperature and out of direct sunlight until transport to the city of Antananarivo, when they were stored at −20°C until extraction.

We extracted swabs in the laboratory at Mahaliana (www.mahaliana.org) in Antananarivo using Qiagen's DNEasy Powersoil kit. We placed swab tips and microbiome buffer into the initial tubes and heated samples for 10 minutes at 60°C prior to bead-beating. We otherwise followed all manufacturer's protocols except we reduced the volume of elution buffer to only 50 μl. We quantified extracts using a QuBit Flurometer with the broad range kit. gDNA concentrations ranged from below the detectable limit to 15.8 ng/μl. Extracts were frozen at −20°C until transport, on ice packs, to the United States where they were placed at −80°C until analysis. We shipped aliquots to Argonne National Laboratory (Lemont, IL) for sequencing via their standard methods using the 515F-806R primer set, 150 × 150 bp, paired-end reads, and Illumina's MiSeq platform.

Bioinformatics and statistical analyses

We processed raw reads using an established bioinformatics pipeline implemented in QIIME2 (Bolyen *et al.* 2019). Sequences were imported into the QIIME2 environment and demultiplexed using version 2019.4. We denoised reads via DADA2 using default parameters and removed low-quality, singleton, and chimeric reads using version 2021.1. Despite the relatively low gDNA yields in our extracted samples, only one sample, from individual "Sa" in the posthibernation season, returned few useable reads (n = 7343) and was subsequently removed from downstream analyses. All other samples were represented by 23,605–69,817 high-quality reads/sample and were retained for analysis.

From filtered reads, we binned sequences into Amplicon Sequence Variants (ASVs) based on 99% sequence similarity. We retained ASVs that were present in minimally two samples. ASV taxonomy was assigned by comparison to the SILVA database (version 138.1) using the pretrained Naïve Bayes classifier for the 515–806 region (Quast *et al.* 2012). We collapsed our ASV tables at phylum and genus level resolution for statistical analyses and data visualizations. We determined the microbial taxa that were present in only one season. From the subset of microbes present in minimally two seasons, we used Linear Discriminant Analysis Effect Size (LEfSe) to determine if there was a significant difference in relative abundances across seasons (Segata *et al.* 2011). To account for multiple testing, we applied the Benjamini–Hochberg correction factor (Benjamini and Hochberg 1995).

We calculated metrics of alpha and beta diversity using QIIME2, rarefying to 20 000 reads per sample at the time of metric computation. For alpha diversity, we calculated measures of microbiome richness (Observed ASVs), evenness (the Shannon index), and phylogenetic representation (Faith's Phylogenetic Diversity). All three metrics were normally distributed. We performed three linear mixed models (LMM), computed using the glmmADMB package (version 0.8.3.3; Skaug *et al.* 2016) in Rstudio (version 1.3.959; RStudio Team 2020) with the R software program (version 4.0.2; R Core Team 2020). In each model, the dependent variable was one metric of alpha diversity, the independent variables were sampling season (three categories: fattening, hibernation, and emergence) and host sex (two categories: male and female), and the random variable was individual lemur.

For beta diversity, we calculated unweighted and weighted UniFrac distances, which capture microbiome distances between pairs of samples respectively based on the presence and relative abundance of unique taxa (Lozupone *et al.* 2011). We performed two Permutational Multivariate Analysis of Variance Using Distance ("adonis") using the vegan package (version 2.5-7; Jari Oksanen *et al.* 2020) in which the dependent variable was one metric of UniFrac distance, and the independent variables were sampling season and host sex. We used the pairwise.adonis function *post hoc* to determine the significance of pairwise comparisons across seasons. Lastly, to determine whether variability in microbiome composition across individuals differed between seasons, we retained all pairwise comparisons of UniFrac distances emanating from the same season. We computed Kruskal–Wallis and Dunn's multiple comparison tests in GraphPad Prism (version 9.1.2).

Results

The dwarf lemurs' gut microbiomes primarily comprised well-known phyla, including *Actinobacteriota*, *Bacteroidota* (i.e. *Bacteroidetes*), *Campilobacterota*, *Firmicutes*, *Fusobacteriota*, and *Proteobacteria* (Fig. 2). There was considerable variation in phylum abundance across individuals within seasons; however, there was also notable variation, on average, across seasons. *Firmicutes* were greatest in the fattening season (41.4%) compared to the hibernation season (24.0%) and after emergence (21.7%). *Bacteroidota* displayed the opposite pattern and were greater in the hibernation season (13.4%) and after emergence (16.3%) compared to the fattening season (3.3%). Both *Actinobacteriota* and *Proteobacteria* were greatest in the hibernation season (11.3% and 46.6%, respectively) compared to the active seasons (1.4%–1.7% and 24%–38%, respectively), while *Campilobacterota* displayed the opposite pattern and was greatest in the active seasons (12.9%–22%) compared to the hibernation season (1.6%). *Fusobacteriota* was greatest after emergence (12.2%) compared to the fattening and hibernation seasons (2.5%–2.6%) and *Cyanobacteria* were only present after emergence and at relatively low abundances (< 1%).

Below phylum-level resolution, we identified 89 microbial taxa in our samples, including 75 identified at genus level, 12 that could not be identified below family level, and one each that could not be identified below order and domain level. Of these 89 taxa, 49 accounted for > 1% of the microbiome in minimally one sample (Fig. 2). These taxa were strongly variable within seasons and across individuals: For example, in the fattening season, *Mycoplasma* accounted for 5.9%–69.7% of the gut microbiome and *Escherichia–Shigella* accounted for 3.2%–50.1% of the gut microbiome. In the hibernation season, unassigned members of the *Enterobacteriaceae* family accounted for 4.1%–69.9% of the gut microbiome, whereas after emergence, *Fusobacterium* accounted for 0.02%–30.9% of the gut microbiome.

Despite these fluctuations, we detected taxa that varied consistently across seasons. Of the 89 identified taxa, 61 (69%) were present in minimally two seasons. In the fattening season compared to the other seasons, the lemurs' gut microbiomes were enriched for *Mycoplasma* (log(LDA) = 5.10, P = .045; Fig. 3A) and trended toward having greater abundances of *Clostridium sensu stricto 1* (log(LDA) = 4.54, P = .058; Fig. 3B). After emergence, the lemurs' microbiomes were enriched for *Campylobacter* (log(LDA) = 5.00, P = .017; Fig. 3C) and *Erysipelatoclostridium* (log(LDA) = 4.11, P = .045; Fig. 3D) and trended toward having greater abundances of *Megamonas* (log(LDA) = 3.96, P = .067). The hibernation season saw the greatest number of significantly enriched taxa compared to the active seasons, including *Corynebacterium* (log(LDA) = 4.18, P = .012; Fig. 4E), *Aerococcus* (log(LDA) = 3.82, P = .045; Fig. 4F), *Staphylococcus* (log(LDA) = 3.74, P = .016; Fig. 4G); unassigned members of the *Enterobacteriaceae* family (log(LDA) = 5.15, P = .017; Fig. 4H); and *Hafnia Obesumbacterium* (log(LDA) = 3.89, P = .045; Fig. 4I). Both *Morganella* (log(LDA) = 4.28, P = .067) and unassigned members of the *Cornybacteriaceae* family (log(LDA) = 3.88, P = .066) trended toward being significantly enriched in the hibernation season compared to the active seasons.

In contrast to the taxa present across seasons, 28 taxa (31%) were only present in one season. A total of 10 of these were present in only one season and in more than 50% of individuals (Fig. 3J–S). No taxon was singularly present in > 50% of individuals in the fattening season. A total of five taxa were singularly present in > 50% of lemurs in the hibernation season and five other taxa were singularly present in > 50% of lemurs after emergence. Most notably, the *Aerococcaceae* genus accounted, on average, for fully 13.8% of the hibernation microbiome (Fig. 3M), while the *Actinomyces* genus and unassigned members of the *Actinomycetaceae* family accounted for another 5.2% of the hibernation microbiome (Fig. 3J and K). All these taxa were universally undetectable in the gut microbiomes of active lemurs.

The diversity of the lemurs' gut microbiome varied by season, increasing across the calendar year from the fattening season to

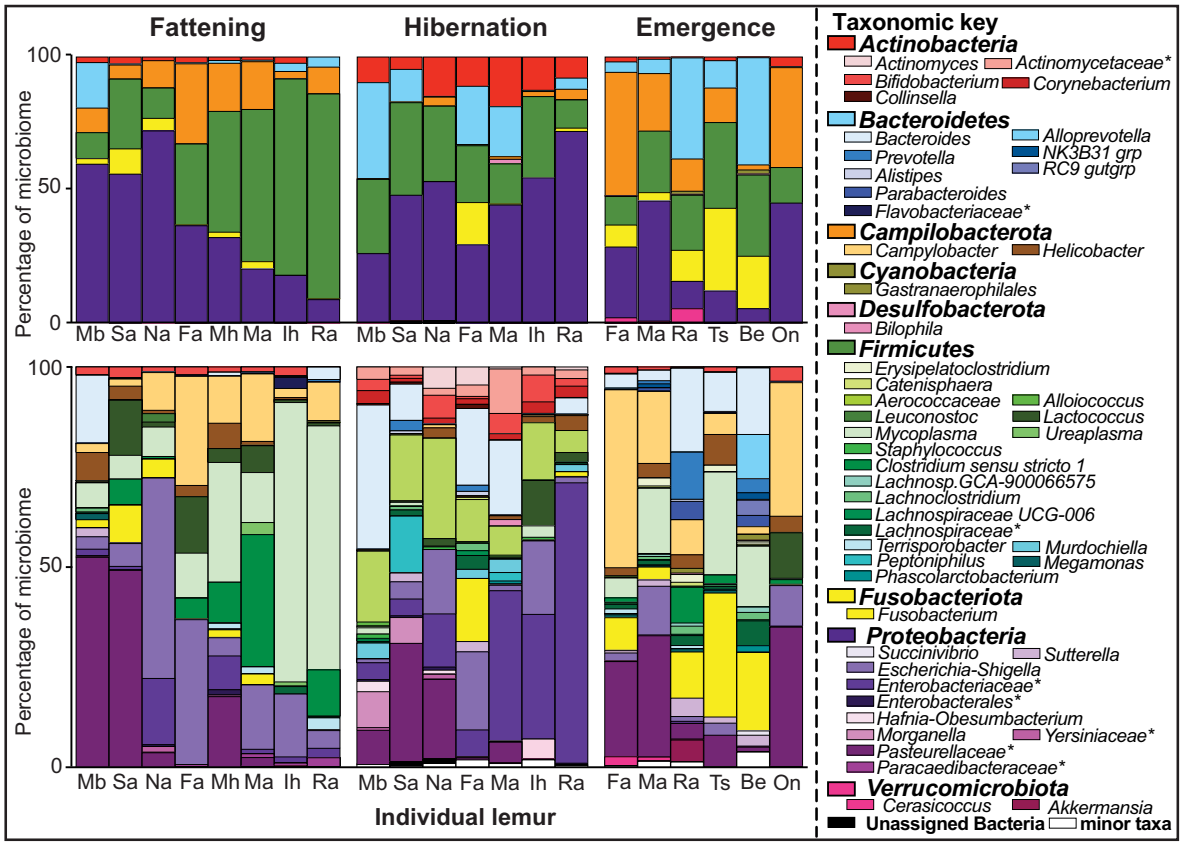

Figure 2. Stacked bar charts depicting microbiome membership at (top) phylum and (bottom) genus level resolution for each lemur sampled in the fattening season, hibernation season, and at emergence from hibernation. Color families reflect unique microbial phyla and distinct shades reflect microbial genera. The taxonomic key is provided to the right. Included are all microbial taxa that accounted for > 1% of the total microbiome in minimally one sample. Asterisks (*) indicate microbial families for whom genus level resolution was unassignable. Unassigned Bacteria refer to all sequences that could not be identified below domain level resolution and minor taxa refers to the summation of all taxa that failed to reach the 1% cutoff.

after emergence. Specifically, alpha diversity was lower in the fattening season compared to the hibernation season as captured by microbiome richness (LMM: $z = -5.10$, $P < .001$; fig. 4A) and evenness (LMM: $z = -2.41$, $P = .016$; fig. 4B), and was modestly lower in the fattening vs. hibernation season as captured by phylogenetic representation (LMM: $z = -1.76$, $P = .08$; fig. 4C). Alpha diversity was likewise lower in the fattening season vs. after emergence as captured by microbiome richness (LMM: $z = -9.50$, $P < .001$), evenness (LMM: $z = -2.92$, $P = .004$), and phylogenetic representation (LMM: $z = -6.30$, $P < .001$). Alpha diversity in the hibernation season vs. after emergence was lower in both microbiome richness (LMM: $z = -5.66$, $P < .001$) and phylogenetic representation (LMM: $z = -4.88$, $P < .001$), although community evenness was equivalent between the hibernation season and after emergence season (LMM: $z = -0.74$, $P = .46$). For no metric was host sex a significant predictor of microbiome diversity (LMM: $z < 1.37$, $P > .17$ for all metrics).

Beta diversity also varied seasonally, indicating that the overall presence and relative abundance of microbial taxa shifted within and between seasons. Specifically, sampling season was associated with both unweighted (PERMANOVA: $F_{2,20} = 4.616$; $R^2 = 0.328$, $P < .001$; Fig. 5A and B) and weighted (PERMANOVA: $F_{2,20} = 3.399$; $R^2 = 0.276$, $P = .002$; Fig. 5C and D) UniFrac metrics and respectively accounted for 33% and 28% of the variance across samples. *Post hoc* pairwise comparisons clarified that all three seasons were significantly different from each other for the unweighted metric ($P < .05$ for all comparisons), whereas for the weighted metric, the hibernation season differed from either active season ($P < .02$ for both comparisons), but the two active seasons did not differ from each other ($P = .87$). Sex was an additional significant predictor of microbiome composition for the unweighted metric (PERMANOVA: $F_{1,20} = 1.941$; $R^2 = 0.069$, $P = .05$), but not the weighted metric (PERMANOVA: $F_{1,20} = 0.861$; $R^2 = 0.035$, $P = .5$).

The lemurs' gut microbiomes were equally variable across individuals within the fattening season, hibernation season, and after emergence when analyzed using the unweighted metric (Kruskal–Wallis: $H = 0.774$, $P = .68$; Fig. 5E). However, for the weighted metric, there was significant variation in interindividual variability between seasons (Kruskal–Wallis: $H = 11.43$, $P = .003$). *Post hoc* tests clarified that there was considerably less interindividual variation in the hibernation season compared to either the fattening season ($P = .024$) or after emergence ($P = .005$; Fig. 5E).

Discussion

In the present study, we highlight changes in gut microbial diversity, variability, and membership linked to the annual hibernation cycle in wild dwarf lemurs. Consistent with our predictions based on hibernating bears and squirrels (Sommer *et al.* 2016, Carey and Assadi-Porter 2017), dwarf lemur consortia showed clear tradeoffs between *Firmicutes* and *Bacteroidetes*, two bacterial phyla that are ubiquitously present in mammalian consortia and relate to the availability of plant substrates. Nevertheless, the microbial genera that contributed to seasonal patterns in dwarf lemurs differed

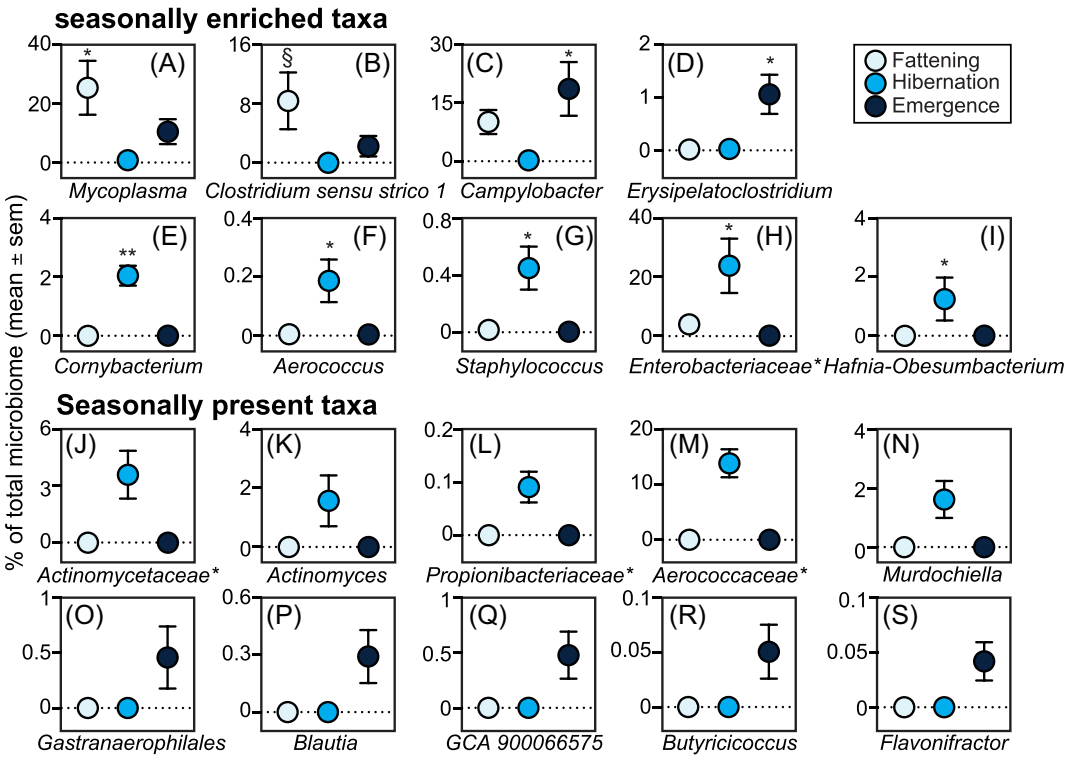

Figure 3. Microbial taxa that were (a)–(i) seasonally enriched or (j)–(s) seasonally present across lemurs in the fattening season (light blue), hibernation season (blue), and at emergence from hibernation (dark blue). Each box represents the relative abundance (%) of a distinct microbial genus with taxon name below; families with asterisks (*) denote families for whom genus level resolution was unassignable. Seasonally enriched taxa (a)-(i) were present in samples from multiple seasons. Seasonally present taxa (j)–(s) only include microbes that were detected in > 50% of individuals within one season. § $P < .10$; * $P < .05$, and ** $P < .01$.

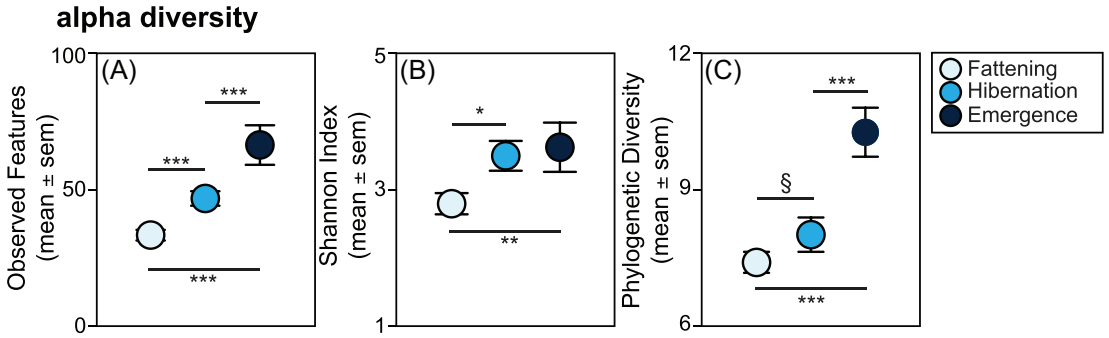

Figure 4. Gut microbial alpha diversity in the fattening season (light blue), hibernation season (blue), and at emergence from hibernation (dark blue). Depicted here are microbial richness, evenness, and phylogenetic breadth as captured by (a) observed features, (b) the Shannon index, and (c) Faith's phylogenetic diversity. § $P < .10$; * $P < .05$, ** $P < .01$; and *** $P < .001$.

from those reported in other hibernators: We noted no increase in *Akkermansia* during hibernation, but rather found blooms in the *Actinomycetaceae* and *Aerococcaceae* families, along with concurrent reductions in *Campylobacter* and *Helicobacter*. Unusually, microbial diversity was not depleted during hibernation, but rather was reduced during fattening, intermediate during hibernation, and greatest at emergence. Thus, we add to the growing literature demonstrating that convergence on an ecological strategy need not be associated with convergence on a singular gut microbiome (Greene *et al.* 2019, Donohue *et al.* 2022). Our results suggest that dwarf lemurs harbor a "primate" gut microbiome stemming from their evolutionary history within the strepsirrhine clade, i.e. tuned to their specific metabolic, digestive, and physiological strategies.

For example, reduced diversity in the gut microbiome prior to hibernation may be explained by dwarf lemurs fattening on fruit sugars (Fietz and Ganzhorn 1999). Within wild lemur species, fruit consumption is correlated to reduced gut microbiome diversity across seasons (Springer *et al.* 2017, Murillo *et al.* 2022). In model systems, high-sugar diets are likewise associated with reduced gut microbiome diversity (Turnbaugh *et al.* 2008b, Sen *et al.* 2017), and with weight gain and obesity (Stanhope 2016, San-Cristobal *et al.* 2020). Most sugars are readily absorbed by epithelial cells in the host's small intestine with fewer resources reaching the lower gut to sustain a rich assemblage of fermentative microbes (Di Rienzi and Britton 2020). Reduced dietary fiber may also alter gut transit time (Müller *et al.* 2018) and constrain diversity in the lower

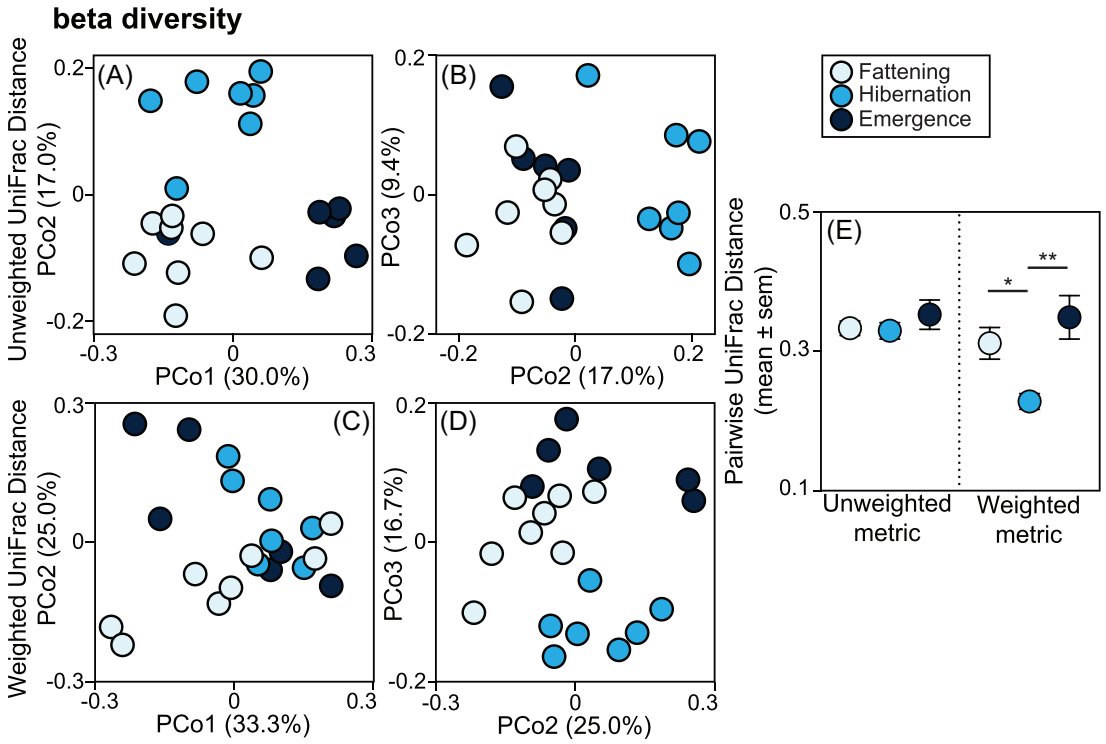

Figure 5. Gut microbial beta diversity in the fattening season (light blue), hibernation season (blue), and at emergence from hibernation (dark blue). Depicted here are metrics of (a) and (b) unweighted and (c) and (d) weighted UniFrac distances graphed in Principal Coordinate (PCo) space and (e) pairwise comparisons between individual lemurs within seasons. * $P < .05$ and ** $P < .01$.

gut. In contrast to the fattening season, the substrates available to gut microbes during hibernation switch to endogenous products, and at emergence from hibernation, dwarf lemurs forage for a generalized diet comprising flowers, insects, and unripe fruits. That gut microbial diversity in dwarf lemurs seemingly mirrors the diversity of available substrates in the gut is reminiscent of nonhibernating mammals and other lemurs: In these systems, gut microbial diversity tracks the host's dietary diversity (Heiman and Greenway 2016, Greene *et al.* 2018, McManus *et al.* 2021), with feeding generalists harboring more diverse microbiotas than do feeding specialists (Dill-McFarland *et al.* 2016, Youngblut *et al.* 2019, Greene *et al.* 2021b). Taken together, these results provide support for the hypothesis that substrate diversity, stemming from dietary intakes or endogenous production, regulates microbial diversity in the lower gut.

The dwarf lemurs' high-sugar diets may also select for fewer microbial members at great abundances that play a specific role in fattening, like *Mycoplasma*. Although often associated with pathogenicity in animals (Rosengarten *et al.* 2000), *Mycoplasma* are gaining recognition for their beneficial functions in the guts of some animals (Rasmussen *et al.* 2021). Due to their small genomes, *Mycoplasma* are metabolically limited; however, many species depend on sugar fermentation as the primary mechanism of ATP generation (Arraes et al. 2007). Importantly, our phylogenetic assignation placed *Mycoplasma* within the *Bacilli* class and *Firmicutes* phylum, whereas other (or older) phylogenies routinely place *Mycoplasma* within the *Mollicutes* class and *Tenericutes* phylum. Such discrepancies highlight ongoing revisions to microbial classifications, as well as the challenge of directly comparing microbial features and functions across studies. While being cautious in our interpretation, we note that there is significant literature linking *Mollicutes* to high-sugar (and high-fat) diets and/or obesity in humans (Crovesy *et al.* 2020) and model systems (Turnbaugh *et al.* 2008a, 2008b, Sen *et al.* 2017). Specifically, germ-free mice colonized with conventional microbiomes and fed westernized diets show blooms in *Mollicutes* (albeit from the *Eubacterium* genus) that outcompete *Bacteroidetes* (Turnbaugh *et al.* 2008b). These *Mollicute*-dominant consortia are even positively associated to host adiposity, suggesting a causal relationship between sugary diets, gut *Mollicutes*, and host fattening (Turnbaugh *et al.* 2008b). *Mollicutes*, including *Mycoplasma*, may be exceptionally good at scavenging simple sugars, like fructose and glucose, from the gut environment, enabling their proliferation under high-sugar conditions. That dwarf lemurs, but not other hibernators, show microbiome features during fattening that are putatively akin to obese humans and model systems, highlights their potential utility as a system for further studies of microbiome-mediated metabolism in primates (Blanco *et al.* 2018).

If sugary diets explain some of the gut microbiome features in dwarf lemurs during the active seasons, the lack of ingesta coupled with warmer body temperatures can explain some of the gut microbiome features we identified during hibernation. Unlike the active seasons, when substrates for microbial metabolism vary due to individual differences in foraging behavior, the substrates available to microbes during hibernation, such as mucin, glycans, and sloughed epithelial cells, are more consistent across individual hosts. This seasonal difference in substrate variability might explain why dwarf lemurs in our study harbored more homogenized consortia (as captured by weighted UniFrac distances) across individuals during hibernation than in either active season. In addition, dwarf lemurs at Tsinjoarivo hibernate at ~15°C (Blanco *et al.* 2013; Fig. 1), which is warm enough to support growth and metabolism by many gut microbes (Khakisahneh *et al.* 2020). The temperature differential between hibernating dwarf lemurs

and Arctic hibernators (that torpor at near-freezing temperatures) could perhaps help account for the microbial diversity in the lemurs' consortia during hibernation. During hibernation, lemurs periodically increase metabolism during short arousals when animals rewarm to euthermia. Arousals are critical to maintaining organ function and may also serve to maintain gut microbiome diversity and composition. During arousals, gut microbes may provide a source of nutritious short-chain fatty acids to their hosts (Stevenson *et al*. 2014), helping to recoup energetic losses accrued as lipid reserves are depleted. Although the lemurs in this study were all sampled during early arousal, future studies could beneficially examine gut microbiome features, including short-chain fatty acid concentrations, in dwarf lemurs during torpor, early and late arousal.

During the hibernation season, we found that dwarf lemurs harbored more microbes at similar relative abundances. This pattern was partially driven by the specific and consistent enrichment across all individuals for the *Actinomycetaceae* and *Aerococcaceae* families, respectively from the *Actinobacteria* and *Firmicutes* phyla. In our study, both the *Aerococcaceae* and *Actinomycetaceae* families were virtually undetectable during the active seasons but bloomed to respectively account for 14% and 5% of the lemurs' gut microbiomes during hibernation. These results are particularly intriguing because they do not match known patterns from other hibernating mammals in which the parent lineages of these families either do not vary seasonally or are more abundant in the active seasons (Carey *et al*. 2013, Stevenson *et al*. 2014, Sommer *et al*. 2016). The *Actinomycetaceae* bloom can perhaps be explained by their capacity to metabolize endogenous products (Ravcheev and Thiele 2017). If true, *Actinomycetaceae* may simply outcompete *Akkermansia* in this system.

The *Aerococcaceae* bloom is more challenging to explain. *Aerococcaceae* is an understudied family of lactic-acid bacteria within the *Lactobacillales* order (König and Fröhlich 2017). Lactic-acid bacteria are generally known for their capacity to ferment sugars into lactate (König and Fröhlich 2017) and for their probiotic roles more broadly (Evivie *et al*. 2017). One possibility relates to increasing gut acidity, as *Lactobacillales* are tolerant to acidic conditions (König and Fröhlich, 2017). Gastric acid may be secreted during arousals but remain unprocessed as it flows through the gut, lowering pH in the colon and boosting abundances of lactic-acid bacteria (O'May *et al*. 2005). Alternately, microbial activity during hibernation, rooted in anaerobic fermentation under hypoxic conditions, could increase concentrations of short-chain fatty acids, which in turn, would also lower pH (Wong *et al*. 2006). This could be especially true if accumulated short-chain fatty acids are more slowly absorbed by metabolically depressed epithelial or microbial cells. Future studies could help clarify if *Aerococcaceae* bloom in this system in response to acidity by testing colonic pH and short-chain fatty acid profiles across seasons.

Although our sample sizes are small and render our findings preliminary, our results nevertheless highlight that the annual hibernation cycle influences gut microbial features in different ways across host species. For dwarf lemurs, seasonal changes in microbiome membership and diversity deviated from temperate hibernators to better mimic the microbial shifts associated with seasonal diets in wild primates (Springer *et al*. 2017, Murillo *et al*. 2022), and obesity and westernized diets in humanized models (Turnbaugh *et al*. 2008a, 2008b). Future studies could characterize the functional consequences of host hibernation to dwarf lemur gut microbiomes using germ-free models (e.g. Sommer *et al*. 2016) and anaerobic culturing. Studies of captive dwarf lemurs undergoing hibernation will also be beneficial (Blanco *et al*. 2021), as they would allow for closer animal monitoring, more frequent sampling of targeted individuals, nonharmful dietary experimentation, and increased sample size (Blanco *et al*. 2022). Integrating data on the lemurs' circulating metabolomes (e.g. D'Alessandro *et al*. 2017) and intestinal transcriptomes (e.g. Sun *et al*. 2020) could better illuminate interaction between microbial and host metabolism during active and torpid states. Ultimately, we suggest that dwarf lemurs offer significant promise and potential as an emerging animal model of gut microbiomes, dietary repertoires, and metabolic strategies under contrasting conditions.

Authors' contributions

L.K.G. and M.B.B. conceived of and designed the study, with help from A.D.Y. L.K.G., M.B.B., and A.D.Y. secured the funding. M.B.B. and J.B.A. performed the field work, with help from L.K.G. L.K.G., M.B.B., J.B.A., and H.A.R. performed the laboratory work. L.K.G. and M.B.B. completed the bioinformatic and statistical analyses. L.K.G. and M.B.B. cowrote the manuscript with input from all authors.

Acknowledgments

We thank the guides, camp agents, and logistical assistants at the Tsinjoarivo Forest, especially Noel Rakotoniaina, Jules Rafalimanatsoa, Nirina Razanadrakoto, Narcisse Rahajanitina, and Jean-Luc Raharison. We thank Professor Zafimahery Rakotomalala and the Department of Animal Biodiversity at the University of Antananarivo for help securing permits. We thank Dr Fidisoa Rasambainarivo and Elizabeth Toomey from the Mahaliana Lab for providing laboratory space and support (www.mahaliana.org). Josh and Judy Greene facilitated the sample storage during transit. Fruitful conversations with Erin McKenney and Sheena Scruggs led to the project's inception. Sarah Owens and the Argonne National Laboratory provided timely sequence data. This is Duke Lemur Center Publication #1508.

Funding

This work was supported by a Duke Microbiome Center Grant-In-Aid for Microbiome Science (to M.B.B., L.K.G., and A.D.Y.) and by the Duke Lemur Center.

Conflicts of interest statement. None declared

References

Arraes FBM, de Carvalho MJA, Maranhão AQ *et al*. Differential metabolism of *Mycoplasma* species as revealed by their genomes. *Genet Mol* 2007;**30**:182–9.

Barnes BM, Buck CL. Hibernation in the extreme: burrow and body temperatures, metabolism, and limits to torpor bout length in arctic ground squirrels. In: Heldmaier G, Klingenspor M, Klaus S (eds.), *Life in the Cold*. Berlin: Springer, 2000, 65–72.

Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol* 1995;**57**:289–300.

Blanco MB, Dausmann KH, Faherty SL *et al*. Tropical heterothermy is "cool": The expression of daily torpor and hibernation in primates. *Evolut Anthropol Issues News Rev* 2018;**27**:147–61.

Blanco MB, Dausmann KH, Ranaivoarisoa JF *et al*. Underground hibernation in a primate. *Sci Rep* 2013;**3**:1–4.

Blanco MB, Greene LK, Ellsaesser L *et al*. Of fruits and fats: white adipose tissue profiles in captive dwarf lemurs are affected

by diet and temperature. *Proc R Soc B* 2022;**289**:1976. DOI: 10.1098/rspb.2022.0598.

Blanco MB, Greene LK, Schopler R *et al.* On the modulation and maintenance of hibernation in captive dwarf lemurs. *Sci Rep* 2021;**11**: 1–11.

Blanco MB, Rahalinarivo V. First direct evidence of hibernation in an eastern dwarf lemur species (*Cheirogaleus crossleyi*) from the high-altitude forest of Tsinjoarivo, central-eastern Madagascar. *Naturwissenschaften* 2010;**97**:945–50.

Bolyen E, Rideout JR, Dillon MR *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;**37**:852–7.

Boyer BB, Barnes BM. Molecular and metabolic aspects of mammalian hibernation: expression of the hibernation phenotype results from the coordinated regulation of multiple physiological and molecular events during preparation for and entry into torpor. *Bioscience* 1999;**49**: 713–24.

Brown A, Lynch D, Bouevitch A *et al.* OMNIgene®• GUT provides easy self-collection and stabilization of liquid fecal samples for microbiome profiling. 2018-10-03. Ottawa: DNA Genotek, 2018. https://www.dnagenotek.com/us/pdf/PD-WP-00056.pdf(31 January 2022, date last accessed).

Carey HV, Andrews MT, Martin SL. Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiol Rev* 2003;**83**:1153–81.

Carey HV, Assadi-Porter FM. The hibernator microbiome: host-bacterial interactions in an extreme nutritional symbiosis. *Annu Rev Nutr* 2017;**37**:477–500.

Carey HV, Walters WA, Knight R. Seasonal restructuring of the ground squirrel gut microbiota over the annual hibernation cycle. *Am J Physiol Regul Integr Compar Physiol* 2013;**304**:R33–R42.

Crovesy L, Masterson D, Rosado EL. Profiles of the gut microbiota of adults with obesity: a systematic review. *Eur J Clin Nutr* 2020;**74**:1251–62.

D'Alessandro A, Nemkov T, Bogren LK *et al.* Comfortably numb and back: plasma metabolomics reveals biochemical adaptations in the hibernating 13-lined ground squirrel. *J Proteome Res* 2017;**16**:958–69.

Dahl WJ, Mendoza DR, Lambert JM. Diet, nutrients and the microbiome. *Prog Mol Biol Transl Sci* 2020;**171**:237–63.

Dausmann K, Glos J, Heldmaier G. Energetics of tropical hibernation. *J Comp Physiol B* 2009:**179**:345–57.

Dausmann K. Flexible patterns in energy savings: heterothermy in primates. *J Zool* 2014;**292**:101–11.

Dausmann KH, Blanco MB. Possible causes and consequences of different hibernation patterns in *Cheirogaleus* species: Mitovy fatsy sahala. In: Lehman SM, Radespiel U, Zimmermann E (eds.), *Dwarf and Mouse Lemurs of Madagascar: Biology, Behavior and Conservation Biogeography of the Cheirogaleidae*. Cambridge: Cambridge University Press, 2016, 335–49.

Dausmann KH, Glos J, Ganzhorn JU *et al.* Hibernation in the tropics: lessons from a primate. *J Comp Physiol B* 2005;**175**:147–55.

Di Rienzi SC, Britton RA. Adaptation of the gut microbiota to modern dietary sugars and sweeteners. *Adv Nutr* 2020;**11**:616–29.

Dill-McFarland KA, Weimer PJ, Pauli JN *et al.* Diet specialization selects for an unusual and simplified gut microbiota in two-and three-toed sloths. *Environ Microbiol* 2016;**18**:1391–402.

Donohue ME, Rowe AK, Kowalewski E *et al.* Significant effects of host dietary guild and phylogeny in wild lemur gut microbiomes. *ISME Commun* 2022;**2**:33.

Evivie SE, Huo G-C, Igene JOm Bian X. Some current applications, limitations and future perspectives of lactic acid bacteria as probiotics. *Food Nutr Res* 2017;**61**:1318034.

Fietz J, Dausmann K. Big is beautiful: fat storage and hibernation as a strategy to cope with marked seasonality in the fat-tailed dwarf lemur (*Cheirogaleus medius*). In: Gould L, Sauther ML (eds.), *Lemurs: Ecology and Adaptation*. Boston: Springer, 2006, 97–110.

Fietz J, Ganzhorn JU. Feeding ecology of the hibernating primate *Cheirogaleus medius:* how does it get so fat?. *Oecologia* 1999;**121**:157–64.

Fietz J, Tataruch F, Dausmann K *et al.* White adipose tissue composition in the free-ranging fat-tailed dwarf lemur (*Cheirogaleus medius;* primates), a tropical hibernator. *J Comp Physiol B* 2003;**173**:1–10.

Geiser F, Ruf T. Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiol Zool* 1995:**68**:935–66.

Greene LK, Blanco MB, Rambeloson E *et al.* Gut microbiota of frugo-folivorous sifakas across environments. *Anim Microbiome* 2021a;**3**:39.

Greene LK, Bornbusch SL, McKenney EA *et al.* The importance of scale in comparative microbiome research: new insights from the gut and glands of captive and wild lemurs. *Am J Primatol* 2019;**81**:e22974.

Greene LK, McKenney EA, O'Connell TM *et al.* The critical role of dietary foliage in maintaining the gut microbiome and metabolome of folivorous sifakas. *Sci Rep* 2018;**8**:14482.

Greene LK, Rambeloson E, Rasoanaivo HA *et al.* Gut microbial diversity and ecological specialization in four sympatric lemur species under lean conditions. *Int J Primatol* 2021b;**42**:961–79.

Heiman ML, Greenway FL. A healthy gastrointestinal microbiome is dependent on dietary diversity. *Mol Metab* 2016;**5**:317–20.

Hill V, Florant G. Patterns of fatty acid composition in free-ranging yellow-bellied marmots (*Marmota flaviventris*) and their diet. *Can J Zool* 1999;**77**:1494–503.

Huus KE, Ley RE. Blowing hot and cold: body temperature and the microbiome. *Msystems* 2021;**6**:e00707–00721.

Johnson GC. Firmicutes and Bacteroidetes explain mass gain variation in an obligate hibernator. Masters Thesis, UCLA, 2021.

Khakisahneh S, Zhang X-Y, Nouri Z *et al.* Gut microbiota and host thermoregulation in response to ambient temperature fluctuations. *Msystems* 2020;**5**:e00514–00520.

König H, Fröhlich J. Lactic acid bacteria. In: König H, Unden G, Fröhlich J (eds.), *Biology of Microorganisms on Grapes, in Must and in Wine*. New York: Springer International Publishing, 2017, 3–41.

Ley RE, Hamady M, Lozupone C *et al.* Evolution of mammals and their gut microbes. *Science* 2008;**320**:1647–51.

Lozupone C, Lladser ME, Knights D *et al.* UniFrac: an effective distance metric for microbial community comparison. *ISME J* 2011;**5**:169.

McManus N, Holmes SM, Louis EE *et al.* The gut microbiome as an indicator of habitat disturbance in a critically endangered lemur. *BMC Ecol Evol* 2021;**21**:1–16.

Mokdad AH, Ford ES, Bowman BA *et al.* Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 2003;**289**: 76–9.

Müller M, Canfora EE, Blaak EE. Gastrointestinal transit time, glucose homeostasis and metabolic health: modulation by dietary fibers. *Nutrients* 2018;**10**:275.

Munro D, Thomas DW. The role of polyunsaturated fatty acids in the expression of torpor by mammals: a review. *Zoology* 2004;**107**: 29–48.

Murillo T, Schneider D, Fichtel C *et al.* Dietary shifts and social interactions drive temporal fluctuations of the gut microbiome from wild redfronted lemurs. *ISME Commun* 2022;**2**:3.

Nishida AH, Ochman H. Rates of gut microbiome divergence in mammals. *Mol Ecol* 2018;**27**:1884–97.

O'May GA, Reynolds N, Macfarlane GT. Effect of pH on an in vitro model of gastric microbiota in enteral nutrition patients. *Appl Environ Microbiol* 2005;**71**:4777–83.

Oksanen J, Blanchet FG, Friendly M *et al.* vegan: community ecology package. 2020, R package version 25-7. https://CRAN.R-project.org/package=vegan. (1 January 2022, date last accessed).

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2012;**41**:D590–D596.

Rasmussen JA, Villumsen KR, Duchêne DA *et al.* Genome-resolved metagenomics suggests a mutualistic relationships between *Mycoplasma* and salmonid hosts. *Commun Biol* 2021;**4**:579.

Ravcheev DA, Thiele I. Comparative genomic analysis of the human gut microbiome reveals a broad distribution of metabolic pathways for the degradation of host-synthetized mucin glycans and utilization of mucin-derived monosaccharides. *Front Genet* 2017;**8**:111.

Rojas CA, Ramirez-Barahona S, Holekamp KE *et al.* Host phylogeny and host ecology structure the mammalian gut microbiota at different taxonomic scales. *Anim Microbiome* 2021;**3**:33.

Rosengarten R, Citti C, Glew M *et al.* Host-pathogen interactions in mycoplasma pathogenesis: virulence and survival strategies of minimalist prokaryotes. *Int J Med Microbiol* 2000;**290**:15–25.

RStudio Team. RStudio: integrated development for R. 2020, Boston: RStudio, Inc.

San-Cristobal R, Navas-Carretero S, Martínez-González MÁ *et al.* Contribution of macronutrients to obesity: implications for precision nutrition. *Nat Rev Endocrinol* 2020;**16**:305–320.

Segata N, Izard J, Waldron L *et al.* Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12**:R60.

Sen T, Cawthon CR, Ihde BT *et al.* Diet-driven microbiota dysbiosis is associated with vagal remodeling and obesity. *Physiol Behav* 2017;**173**:305–17.

Sepulveda J, Moeller AH. The effects of temperature on animal gut microbiomes. *Front Microbiol* 2020;**11**:384.

Singh RK, Chang H-W, Yan D *et al.* Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017;**15**:73.

Skaug H, Fournier D, Bolker B *et al.* Generalized linear mixed models using AD Model Builder. 2016, R package version 0.8.3.3.

Sommer F, Ståhlman M, Ilkayeva O *et al.* The gut microbiota modulates energy metabolism in the hibernating brown bear *Ursus arctos*. *Cell Rep* 2016;**14**:1655–61.

Sonoyama K, Fujiwara R, Takemura N *et al.* Response of gut microbiota to fasting and hibernation in Syrian hamsters. *Appl Environ Microbiol* 2009;**75**:6451–6.

Springer A, Fichtel C, Al-Ghalith GA *et al.* Patterns of seasonality and group membership characterize the gut microbiobiota in a longitudinal study of wild Verreaxu's sifakas (*Propithecus verreauxi*). *Ecol Evol* 2017;**7**:5732–45

Stanhope KL. Sugar consumption, metabolic disease and obesity: the state of the controversy. *Crit Rev Clin Lab Sci* 2016;**53**:52–67.

Stevenson TJ, Duddleston KN, Buck CL. Effects of season and host physiological state on the diversity, density, and activity of the arctic ground squirrel cecal microbiota. *Appl Environ Microbiol* 2014;**80**:5611–22.

Sun H, Wang J, Xing Y *et al.* Gut transcriptomic changes during hibernation in the greater horseshoe bat (*Rhinolophus ferrumequinum*). *Front Zool* 2020;**17**:21

Tøien Ø, Blake J, Edgar DM *et al.* Hibernation in black bears: independence of metabolic suppression from body temperature. *Science* 2011;**331**:906–9.

Turnbaugh PJ, Bäckhed F, Fulton L *et al.* Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008b;**3**:213–23.

Turnbaugh PJ, Backhed F, Fulton L *et al.* Marked alterations in the distal gut microbiome linked to diet-induced obesity. *Cell Host Microbe* 2008a;**3**:213.

Wong JM, De Souza R, Kendall CW *et al.* Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* 2006;**40**:235–43.

Xiao G, Liu S, Xiao Y *et al.* Seasonal changes in gut microbiota diversity and composition in the greater horseshoe bat. *Front Microbiol* 2019;**10**:2247.

Youngblut ND, Reischer GH, Walters W *et al.* Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. *Nat Commun* 2019;**10**:2200.