

## RESEARCH ARTICLE

# Minerals in the foods and diet of diademed sifakas: Are they nutritional challenges?

Mitchell T. Irwin<sup>1,2</sup> | Jean-Luc Raharison<sup>2,3</sup> | Colin A. Chapman<sup>4,5</sup> |  
Randall E. Junge<sup>6</sup> | Jessica M. Rothman<sup>7,8</sup>

<sup>1</sup>Department of Anthropology, Northern Illinois University, DeKalb, Illinois

<sup>2</sup>SADABE Madagascar, Antananarivo, Madagascar

<sup>3</sup>Department of Animal Biology, University of Antananarivo, Antananarivo, Madagascar

<sup>4</sup>Department of Anthropology and McGill School of Environment, McGill University, Montreal, Quebec, Canada

<sup>5</sup>Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, New York, New York

<sup>6</sup>Department of Animal Health, Columbus Zoo and Aquarium, Columbus, Ohio

<sup>7</sup>Department of Anthropology, Hunter College, City University of New York, New York, New York

<sup>8</sup>New York Consortium in Evolutionary Primatology (NYCEP), New York, New York

## Correspondence

Mitchell T. Irwin, Department of Anthropology, Northern Illinois University, DeKalb, IL 60115. Email: mirwin@niu.edu

Minerals, though needed in small quantities, are essential to metabolic processes, and deficiencies can seriously threaten health, reproduction and survival. Despite this, few studies have measured mineral composition of wild primate foods and fewer have quantified mineral intake. Here we measured the concentration of nine minerals in 75 foods of diademed sifakas (*Propithecus diadema*; five groups) in habitats with varying levels of disturbance at Tsinjoarivo and estimated daily intakes using focal-animal feeding data and intake rates over one year. For six minerals (Ca, P, Na, Fe, Zn, and Cu), mean concentrations in foods fell short of the National Research Council's (NRC) recommendations for captive primates. Concentrations were highest in lianas, herbs, and epiphytes, and hemiparasites had exceptionally high Na. Leaves tended to have higher concentrations than fruits or flowers, but overlap was extensive. Mineral concentrations in daily diets varied little seasonally, but absolute intakes (g/day) were higher in the abundant season, due to the increase in food ingested. Disturbed habitat groups' diets had higher mineral concentrations for five minerals, but this translated into increased intakes only for Cu, as these groups ate less food overall. Overall, comparisons with percentage-based NRC recommendations suggests deficiencies, but this is contradicted by: (1) the fact that mass-specific intakes exceeded human recommendations, and (2) the lack of observed signs of deficiency. Ongoing efforts to quantify mineral consumption across wild primate populations and better understanding requirements on both a percentage and absolute basis will help in understanding effects on food selection, managing primate habitats and formulating captive diets.

## KEYWORDS

habitat disturbance, lemurs, micronutrients, nutrition, seasonality

## 1 | INTRODUCTION

Animals' dietary requirements include both macronutrients (protein, fat and carbohydrate, required in high quantities) and micronutrients (vitamins and minerals, required in low quantities). Minerals are a unique component of animal diets because they are elements rather than organic molecules, although they often exist in chemical complexes when ingested. Minerals serve various roles in the vertebrate body, including mineralized supportive tissue such as bone, maintaining osmotic gradients for nervous impulse transmission and muscle contraction, and providing structural components for enzymes and other proteins (Barboza, Parker, & Hume, 2009; National Research Council, 2003).

One fundamental assumption of nutritional ecology is that requirements of various nutrients can be selective pressures promoting food preferences and foraging choices. However, rules affecting foraging choices are complex and models used to approximate these generally fall in three categories: maximizing or optimizing intakes of nutrients, balancing nutrients, and limiting the intake of undesired compounds, such as fiber or plant secondary metabolites (PSMs) (Felton, Felton, Lindenmayer, & Foley, 2009). In theory, a foraging individual's "goal" might focus on one limiting nutrient or toxin, or be a balance of different approaches. For example, a folivore might seek to balance maximizing energy intake and minimizing PSMs. Whenever an animal cannot optimize its diet on all axes simultaneously, it should seek "rules of compromise" that minimize the fitness costs of missing

targets (Simpson & Raubenheimer, 2012). These costs include both shortfalls (of energy, macronutrients, vitamins or minerals) and overshooting targets, leading to toxicity in the case of some PSMs and minerals, or simply the cost of excreting excesses.

There is growing evidence that at least some primate foraging choices serve to increase mineral intakes. Primatologists have documented consumption of small amounts of items not traditionally considered food that are high in minerals but not macronutrients: decaying wood (Chaves, Stoner, Angeles-Campos, & Arroyo-Rodriguez, 2011; Rothman, Van Soest, & Pell, 2006), soil (Krishnamani & Mahaney, 2000), and urine and water from mud puddles (Rode, Chapman, Chapman, & McDowell, 2003). More complex is the question of whether mineral needs drive animals' choices of more traditional food types: this may be more easily inferred for otherwise low-quality foods such as bark and petioles (Rode et al., 2003), but mineral content can also be a factor affecting the selection of traditional foods such as fruit, leaves and flowers, both favoring specific foods, and promoting higher dietary diversity due to the complementarity of different foods as mineral sources (Behie & Pavelka, 2012; Cancelliere, DeAngelis, Nkurunungi, Raubenheimer, & Rothman, 2014). Additionally, some studies suggest that minerals limit population growth (Rode, Chapman, McDowell, & Stickler, 2006), or that specific landscape elements, such as localized swamps, may be key mineral sources and thereby disproportionately impact ranging and habitat selection (Magliocca & Gautier-Hion, 2002; Oates, 1978; Rode et al., 2006).

While early studies of primate foods suggested some simple generalizations, including that leaves tended to be richer mineral sources than other plant foods (Janson & Chapman, 1999; Lambert, 2011; but see Nagy & Milton, 1979), newer research has revealed considerable diversity among plant species, differing patterns among specific minerals, and wide variation among different sites and habitat types (Behie & Pavelka, 2012; Cancelliere et al., 2014; Schmidt et al., 2010; Silver, Ostro, Yeager, & Dierenfeld, 2000; Yeager, Silver, & Dierenfeld, 1997). Further research is necessary to better understand minerals' role in primate nutritional ecology, diet selection and health, and quantification of minerals as both a component of foods (percentage basis or mg/kg) and in overall dietary intakes (absolute intakes in g) seems crucial to this process.

In this study we explore the role of minerals in the diet of diademed sifakas. First, we quantify mineral content of foods, testing the prediction that content varies among plant parts (with foliage exhibiting highest levels) and plant types (with non-tree plants exhibiting highest levels (Cancelliere et al., 2014)). Second, we combine food analyses and observational data to estimate proportional and absolute mineral intake and compare these to suggested requirements for primates, testing the prediction that wild intakes fall below requirements. Third, we assess seasonality and habitat differences both in the content of the diet and in absolute mineral intakes. In terms of diet proportions (percent of dry matter), we predicted higher mineral levels in the lean season and in more disturbed habitat (in both situations sifakas are more folivorous); in terms of absolute intakes (g/day), we predicted highest intakes in the abundant season and in less disturbed habitat (in both situations sifakas consume more food).

## 2 | METHODS

### 2.1 | Study Site and Subjects

Tsinjoarivo forest is a mid-altitude forest in eastern central Madagascar. Diademed sifakas (*Propithecus diadema*) have been studied here since 2002 (Irwin, 2008; Irwin, Raharison, Raubenheimer, Chapman, & Rothman, 2015). Here we report on observations of five habituated groups from June 2006 to July 2007: two in relatively undisturbed continuous habitat at Vatazeza ("CONT" groups; 19°43.250S, 47°51.410E; 1,396 m) and three in fragmented, disturbed habitat at Mahatsinjo ("FRAG" groups; 19°40.940S, 47°45.460E; 1,590 m). CONT groups had high values for cumulative basal area/ha of trees >5 cm DBH, a useful correlate of habitat disturbance (Irwin & Raharison, in press), while FRAG4 was intermediate, FRAG2 was low and FRAG3 was very low. Seasonality is marked: the "abundant season," roughly October–March, exhibits higher than average temperature, rainfall and food availability, while the "lean season," roughly April–September, exhibits lower than average levels; detailed description of these seasons is provided elsewhere (Irwin, 2006, 2008). Sifakas mate in December, gestate between December and June/July, give birth in June or July, and lactate until roughly January.

The sifakas are largely folivorous (53% of feeding time on foliage, 24% on fruits, 7% on seeds, 15% on flowers), with an abundant season emphasis on fruits and seeds and a lean season emphasis on leaves and flowers, largely from the fallback food *Bakerella clavata*, a mistletoe (Irwin, 2008). FRAG groups have lower dietary diversity and differ from CONT groups in that their fruits derive largely from *B. clavata* rather than canopy trees. Macronutrient intakes vary considerably throughout the year: abundant season intakes (g of food per day) in CONT groups are up to four times higher than lean season intakes, though the relative contribution of different macronutrients (protein, fat, carbohydrates) is remarkably consistent across seasons; FRAG groups largely lack preferred abundant season fruits and consequently have "lean-season-like" intakes year-round (Irwin, Raharison, Raubenheimer, Chapman, & Rothman 2014; Irwin et al., 2015).

### 2.2 | Observational Data

We collected data in 12 periods, each of 11–21 days (Irwin et al., 2015). These periods were categorized into 5 seasons: 1 (June 16–August 14, 2006; periods 1–2), 2 (October 22–December 19, 2006; periods 3–5), 3 (January 27–April 14, 2007; periods 6–8), 4 (April 27–June 2, 2007; periods 9–10), and 5 (June 18–July 26, 2007; periods 11–12) (Irwin et al., 2015). Seasons 1, 4, and 5 are in the lean season, while seasons 2 and 3 are in the abundant season. The CONT2 and FRAG2 adult females lactated during the first half of the study but were not gestating during the second half; CONT1 and FRAG4 adult females lactated during the first half and gestated during the second half; the FRAG3 adult female did not give birth in 2006 and died shortly after the birth season. The death of both animals in FRAG3 necessitated that group's replacement by FRAG3 in seasons 2–5.

Data were collected on animals  $\geq 2$  years old (at 2 years animals are roughly 70% adult body mass; actual body mass ranged from 4,000 to

5,600 g) at the beginning of the study during all-day focal-animal follows, led by MTI and/or local research assistants. Animals were rarely out of sight (0.2% of 5-min instantaneous records), and there were no obvious differences in visibility among seasons. For each feeding bout, we recorded start and stop time, plant part and species consumed; bouts were stopped when a pause exceeded 10 s. Soil feeding was recorded, but not included in this analysis (0.3% of feeding time). Our sample includes 363 focal-animal days, 18,253 feeding bouts and 1,090 feeding hours divided among 18 individuals (CONT1: 5; CONT2: 4; FRAG2: 3; FRAG3: 2; FRAG4: 4). Representative intake rates samples were collected either using one-minute samples within feeding bouts, recording the number of units consumed (for rapidly consumed items, such as young leaves or flowers; some intervals were shorter, when the bout ended early), or by recording start and stop time for each item, or a subset of items, within bouts (for larger items, usually fruits and seeds). We amassed 16,565 intake records totaling 283 hr across the five groups.

This research complied with protocols approved by McGill University's Animal Care Committee, and adhered to the legal requirements of Madagascar and to the American Society of Primatologists' Principles for the Ethical Treatment of Non Human Primates; research permits were issued in Madagascar by the Ministry of Environment, Water and Forests (#120/06/MINENV.EF/SG/DGEF/DPB/SCBLF/RECH).

### 2.3 | Sample Collection, Chemical Analyses, and Nutrient Calculations

We analyzed the mineral content of 121 plant samples, representing 58 plant species and 75 species-plant part combinations. Parts sampled were flower buds, flowers, fruits without seed (when seeds were spit or dropped), fruit with seed, seed, young leaves, and distal growing shoots. Where possible, samples were collected from plants on which the animals fed; failing that we selected nearby conspecific plants as similar as possible to those used (in terms of size, phenological state, and microhabitat). Samples were processed in the same way as by sifakas, then dried in trays inside a tent pitched in direct sun; all concentrations are expressed as percent dry matter (DM). For analysis, samples were divided into four plant types: herbaceous plants/lianas/epiphytes (i.e., non-parasitic, non-woody plants), parasitic plants (including the holoparasite *Langsdorffia*, and two species of hemiparasitic mistletoe), "edge trees" (those existing at edges or light gaps and never reaching canopy height, and "forest trees" (species typically growing in primary forest, including canopy and understory). Samples were also divided into three food types: flower buds and flowers, fruit and/or seed, and foliage.

Food samples were analyzed for macronutrient content (described elsewhere: (Irwin et al., 2014)) and minerals. Mineral analyses were performed at Dairy One Forage Lab (Ithaca, NY) and assayed Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, and Mo. Samples were ashed in a muffle furnace at 500°C for 4 hr, then ash residue was suspended in 3 ml of 6N HCl and evaporated to dryness on a 100–120°C hot plate. Minerals were then extracted with acid solution (1.5N HNO<sub>3</sub>+ 0.5N HCl) and analyzed using a Thermo Jarrell Ash IRIS Advantage HX Inductively

Coupled Plasma (ICP) Radial Spectrometer. Raw data are found in supporting information. For Mo, 70 of 121 samples fell below the detection threshold of 0.1 ppm and the remaining samples varied from 0.1 to 0.8; these data are presented in the appendix but not included in analyses.

We used the following formula to calculate daily mineral intakes for focal animals:

$$DI_y = \sum_{i=1}^B D_i \times R_x \times M_x \times C_x \times Q_{x,y}$$

where  $DI$  = daily intake of  $y$  (expressed in grams),  $B$  = number of feeding bouts,  $D_i$  = duration of feeding bout  $i$  (sec),  $R_x$  = average intake rate (units/sec) for food  $x$  (plant part/species combination),  $M_x$  = mass per intake unit (g/unit DM) for food  $x$ ,  $C_x$  = intake conversion factor for food  $x$  (for *Bakerella* flowers and *Salacia madagascariensis* seeds), and  $Q_{x,y}$  = concentration of mineral  $y$  in food  $x$  (percent DM). In calculating daily intakes we used average intake rates pooled across individuals and sites, as only quite small differences were detected among groups and individuals (see Irwin et al., 2015 for discussion). For estimates of mass-specific intakes we used individual body mass derived from capture data; these were available for most animals within 2 years of this study (see Irwin, Junge, Raharison, & Samonds, 2010 for further detail).

Due to high dietary diversity, we did not sample all foods; instead we preferentially sampled those foods most represented in overall feeding time. In total, mineral content was available for foods representing 76% of feeding time for CONT groups and 84% of feeding time for FRAG groups. When mineral content for a certain food was unavailable, we substituted data from other samples representing the same plant part and stage. If congeners had been sampled, these values were used; when no congeners were sampled, we used the average for all species for that plant part/stage.

### 2.4 | Analyses

To assess differences among the four plant types and three food types we used Kruskal–Wallis tests (R Core Team, 2015); a two-factor univariate ANOVA was not applied due to unbalanced sample size and lack of representation in some plant type  $\times$  food type combinations. When significant differences were detected, Dunn's pairwise posthoc test (using Holm's correction) was used to compare groups.

We used linear mixed models (LMMs) to assess the effects of site (CONT vs. FRAG), season (lean vs. abundant) and site  $\times$  season interaction on daily intake of all nine minerals, on both a proportional (% DM) and absolute (g/day) basis. Because groups and individuals within them were sampled repeatedly, both group ( $n = 5$ ) and individual nested within group ( $n = 18$ ) were included as random effects (intercept only) in the model. Model fitting followed four steps and used likelihood ratio tests (LRTs; threshold for inclusion  $p < 0.05$ ). First, a fully loaded model (two fixed effects, two nested random effects) was compared to a model with a simplified random effect structure (group only) using LRTs from reduced maximum likelihood (REML) model fitting. The simplified model was adopted if the addition of the individual term did not significantly improve model

fit; otherwise the first model was retained. Second, the effect of the site  $\times$  season interaction on model fit was assessed using maximum likelihood (ML), and the interaction was retained in the model if the LRT was significant. If the interaction was retained, fixed factors were not tested using LRTs individually; otherwise, season and then site were tested (in that order) and retained in the model only if the LRT was significant (ML model fitting). LRTs are reported for all fixed factors tested; for the intercept and all fixed factors retained in the model we report coefficient ( $\pm$ SE),  $t$ -values and associated  $p$ -values; although interpreting these is more complex than LRTs, they can be useful in comparing models (particularly when a significant interaction precludes testing season and site via LRT). Final model results are reported using REML models. LMMs used the nlme package (Pinheiro, Bates, DebRoy, Sarkar, & Team, 2015) in R (R Core Team, 2015).

### 3 | RESULTS

#### 3.1 | Mineral Content of Foods and Variation Among Plant and Food Types

Plant types (herb/liana/epiphyte, parasite, edge tree and forest tree) differed significantly for six of nine minerals assayed (Ca, Mg, Na, Fe, Zn and Cu), but there was little consistency across those six minerals in patterns of variation, and high variability within some categories (Table I; Figure 1). Herbs/lianas/epiphytes were the richest sources of Ca, Mg, Fe, Zn and Cu. Parasitic plants, despite being lower than average for most minerals, had the highest Na values (2–8 times higher than other categories). The fact that only two edge trees were consumed (*Solanum mauritianum* fruit and *Maesa lanceolata* leaves) makes it hard to assess this category with any statistical power, yet they had average or low mineral concentrations compared to other foods, with one exception (*M. lanceolata*'s high Ca: 0.77%).

Food types (flowers, foliage, fruit/seed) differed significantly in seven of nine minerals: foliage had the highest levels for Ca, P, Mg, Zn, and Cu, while fruit/seed was highest for Fe and flowers were highest for Mn. Foliage had above-average concentrations of all minerals except Na, and fruit/seed had below-average concentrations in all but Na and Fe.

#### 3.2 | Dietary Mineral Intakes: Proportional Representation in Diet

In terms of proportional representation in the diet, six of nine minerals were below NRC recommended percentage-based intakes (Ca, P, Na, Fe, Zn, and Cu; overall and in both seasons and all groups), but the other three were above recommended intakes (overall and in both seasons and all groups; Table II).

LMMs for proportional intakes (percentage of DM ingested; Table III) revealed that CONT groups' diets had significantly lower concentrations of five minerals (Ca, P, K, Na, and Mn; comparing intercepts with coefficients shows a 11–52% reduction) and significantly higher concentrations of Cu (56% increase); for the

remainder (Mg, Fe, and Zn) models suggest no effect. The lean season had higher concentrations for two minerals (Ca, P; 19 and 3% increase respectively), lower concentrations for two (Fe, Cu; 49 and 26% decrease respectively), and little impact of season for the remainder.

The difference between CONT and FRAG groups does not cleanly follow the observed differences among food types and plant types. CONT and FRAG groups differed in the contribution of the three main food types (buds/flowers, fruit/seed and foliage) to feeding time but their reliance on foliage, the most mineral-rich food type, was very similar (CONT: 8.1, 42.1, 49.2%; FRAG: 15.8, 31.2, 52.3%). In terms of plant type, the herb/liana/epiphyte category was most mineral-rich, yet CONT groups had a higher proportion of this category in their diet (16.5%) than did FRAG groups (8.9%). FRAG groups tended to rely more on hemiparasites (mistletoe) (FRAG: 31.6%, CONT: 16.8%) and edge trees (FRAG: 12.9%, CONT: 7.5%), yet these two categories were relatively mineral-poor.

#### 3.3 | Dietary Mineral Intakes: Absolute Intakes

LMMs for absolute intakes (Tables IV and V) revealed little impact of site on intakes: CONT groups had higher Cu intakes (146% increase), but site was either excluded from the model or included with a non-significant  $t$ -value for the remaining eight minerals. The effect of season was more consistent, being a significant predictor of intake for all minerals, with lean season intakes being lowest. The magnitude of this effect was high, with a 28–67% lean season reduction. Sifakas' mass-adjusted intakes (Table VI) were considerably higher than recommended human intakes. Finally, Ca:P, Ca:K and Fe:Cu ratios in sifaka diets (Table VII) were close to NRC-recommended ratios, while the remainder were not: Ca:Na (high), Ca:Mg (low), Na:Mg (low), and Zn:Cu (low).

### 4 | DISCUSSION

#### 4.1 | Mineral Content of Sifaka Foods and Comparisons With Other Primates

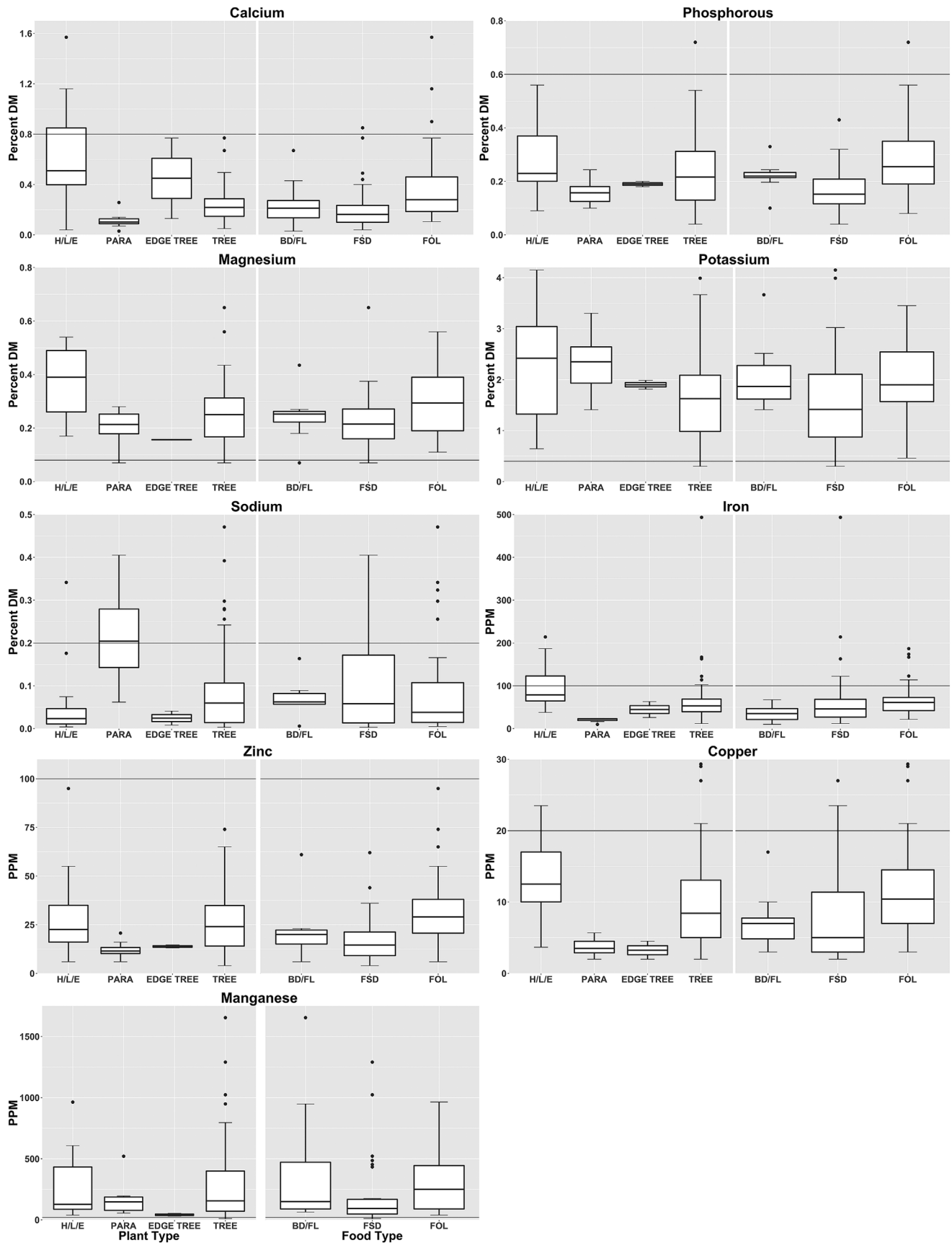
The mineral content of sifaka foods is similar to those of other primates studied to date (Table VIII). Six of the nine minerals assessed were below NRC recommended concentrations, following our prediction (Ca, P, Na, Fe, Zn, and Cu; Table III); this echoes several earlier studies in which primate foods often fall below NRC-recommended levels (Cancelliere et al., 2014; National Research Council, 2003; Rode et al., 2003). As suggested by previous studies, this reinforces the general knowledge that NRC recommendations are conservative, and that wild primate populations are surviving on lower-mineral diets. It is therefore important to be cautious in interpreting low concentrations as evidence that diets are "deficient" in minerals; this inference should not be made unless actual health consequences can be documented. However, these comparisons can serve to focus future research targeting those minerals that seem to be "low."

**TABLE 1** Variation in mineral content of diademmed sifaka foods at Tsinjoarivo, Madagascar

Mineral	Plant type					Food type				
	All foods (75)	Herb/liana/epiphyte (13)	Parasite (8)	Edge tree (2)	Forest tree (52)	Significance <sup>1</sup>	Flowers (8)	Fruit/seed (30)	Foliage (37)	Significance <sup>1</sup>
Ca (%DM)	0.30 ± 0.27	0.60 ± 0.44 <sup>a</sup>	0.12 ± 0.07 <sup>b</sup>	0.45 ± 0.45 <sup>abc</sup>	0.25 ± 0.14 <sup>c</sup>	X <sup>2</sup> = 16.55	0.25 ± 0.20 <sup>ab</sup>	0.22 ± 0.20 <sup>a</sup>	0.38 ± 0.31 <sup>b</sup>	X <sup>2</sup> = 10.49
P (%DM)	0.23 ± 0.12	0.28 ± 0.14	0.16 ± 0.05	0.19 ± 0.01	0.23 ± 0.13	p < 0.001	0.22 ± 0.06 <sup>ab</sup>	0.17 ± 0.09 <sup>a</sup>	0.28 ± 0.14 <sup>b</sup>	p = 0.005
Mg (%DM)	0.27 ± 0.12	0.37 ± 0.13	0.20 ± 0.07 <sup>a</sup>	0.16 ± 0.002 <sup>a</sup>	0.25 ± 0.11 <sup>a</sup>	p = 0.16	0.24 ± 0.10 <sup>ab</sup>	0.23 ± 0.11 <sup>a</sup>	0.30 ± 0.12 <sup>b</sup>	X <sup>2</sup> = 7.09
K (%DM)	1.85 ± 0.89	2.27 ± 1.10	2.31 ± 0.68	1.90 ± 0.12	1.67 ± 0.83	p < 0.004	2.08 ± 0.74	1.63 ± 0.99	1.98 ± 0.81	p = 0.029
Na (%DM)	0.096 ± 0.111	0.061 ± 0.096 <sup>a</sup>	0.213 ± 0.118 <sup>b</sup>	0.025 ± 0.023 <sup>ab</sup>	0.089 ± 0.105 <sup>a</sup>	X <sup>2</sup> = 6.65	0.072 ± 0.044	0.109 ± 0.121	0.091 ± 0.112	X <sup>2</sup> = 4.22
Fe (ppm)	66.2 ± 64.9	99.7 ± 58.9 <sup>a</sup>	20.3 ± 4.9 <sup>b</sup>	44.6 ± 26.3 <sup>ab</sup>	65.7 ± 68.1 <sup>a</sup>	p = 0.08	36.1 ± 19.3 <sup>a</sup>	71.9 ± 91.5 <sup>ab</sup>	68.0 ± 40.1 <sup>b</sup>	p = 0.12
Zn (ppm)	25.0 ± 17.7	30.9 ± 24.4	12.1 ± 4.5	13.8 ± 1.1	26.0 ± 16.4	X <sup>2</sup> = 11.74	22.5 ± 16.6 <sup>ab</sup>	17.6 ± 12.8 <sup>a</sup>	31.6 ± 19.1 <sup>b</sup>	X <sup>2</sup> = 0.94
Cu (ppm)	9.76 ± 6.77	13.0 ± 5.4 <sup>a</sup>	3.69 ± 1.27 <sup>b</sup>	3.25 ± 1.77 <sup>ab</sup>	10.13 ± 7.00 <sup>a</sup>	p < 0.008	7.54 ± 4.37 <sup>ab</sup>	7.76 ± 6.70 <sup>a</sup>	11.86 ± 6.74 <sup>b</sup>	p = 0.001
Mn (ppm) <sup>2</sup>	275 ± 315	269 ± 283	175 ± 150	42 ± 17	300 ± 343	X <sup>2</sup> = 8.58	432 ± 574 <sup>ab</sup>	205 ± 298 <sup>a</sup>	297 ± 242 <sup>b</sup>	X <sup>2</sup> = 10.98
						p < 0.001				p = 0.004
						p = 0.28				X <sup>2</sup> = 7.14
										p = 0.028

<sup>1</sup>Refers to results of a Kruskal–Wallis test; shared superscripts represent statistically indistinguishable categories following Dunn's test with Holm's adjustment for multiple comparisons.

<sup>2</sup>Kruskal–Wallis test indicates significance, yet lowest *p*-value in pairwise comparisons was 0.051 for the parasite/forest tree comparison.



**FIGURE 1** Boxplots depicting dry matter concentration of 9 minerals in 75 diademed sifaka foods at Tsinjoarivo, Madagascar. Sample size for plant type: herb/liana/epiphyte: 13; parasite: 8; edge tree: 2; forest tree: 52. Sample size for food type: flowers: 8; fruit/seed: 30; foliage: 37. Horizontal reference lines indicate recommended values for captive primates (National Research Council, 2003)

**TABLE II** Proportional mineral content in the diet of five diademed sifaka groups at Tsinjoarivo, Madagascar, compared to recommended concentrations

	Recommended dietary concentration <sup>a</sup>	% of daily diet (DM)—combined and by group (CONT1, CONT2/FRAG2, FRAG3, FRAG4)		
		Overall diet	Abundant season	Lean season
Ca (%DM)	0.80	0.24 (0.22, 0.19/0.32, 0.36, 0.24)	0.20 (0.17, 0.14/0.29, -, 0.23)	0.28 (0.26, 0.24/0.35, 0.36, 0.26)
P (%DM)	0.60	0.17 (0.16, 0.16/0.19, 0.19, 0.18)	0.17 (0.16, 0.16/0.18, -, 0.18)	0.18 (0.17, 0.17/0.19, 0.19, 0.18)
Mg (%DM)	0.08	0.23 (0.23, 0.24/0.22, 0.22, 0.23)	0.23 (0.24, 0.24/0.22, -, 0.23)	0.22 (0.21, 0.24/0.22, 0.22, 0.23)
K (%DM)	0.40	1.75 (1.54, 1.46/2.04, 2.08, 2.02)	1.58 (1.32, 1.22/1.99, -, 1.95)	1.90 (1.75, 1.69/2.09, 2.08, 2.09)
Na (%DM)	0.20	0.084 (0.060, 0.073/0.103, 0.122, 0.105)	0.074 (0.052, 0.048/0.106, -, 0.102)	0.093 (0.067, 0.098/0.101, 0.122, 0.108)
Fe (ppm)	100	61 (57, 69/67, 45, 51)	76 (69, 79/96, -, 61)	47 (45, 59/42, 45, 39)
Zn (ppm)	100	17 (16, 17/18, 22, 18)	17 (16, 16/19, -, 18)	17 (16, 17/18, 22, 18)
Cu (ppm)	20	7.6 (8.5, 9.6/5.9, 5.8, 6.4)	8.7 (10.3, 10.4/6.2, -, 7.0)	6.7 (6.7, 8.8/5.6, 5.8, 5.6)
Mn (ppm)	20	193 (152, 157/231, 277, 245)	177 (135, 120/239, -, 237)	209 (169, 194/224, 277, 255)

<sup>a</sup>Recommended Intakes following (National Research Council, 2003).

The differences detected among plant parts and food types (Table I) largely follow predictions and include some that mirror previous studies. For example, leaves had significantly higher concentrations than fruits for six of nine minerals, as previously found in howler monkeys and mountain gorillas (Behie & Pavelka, 2012; Cancelliere et al., 2014; Silver et al., 2000). However, this difference does not hold across all minerals or across all primate diets studied (Rode et al., 2003; Schmidt et al., 2010), and overlap across food types is often considerable. Although statistically significant differences are present, the wide variation within some categories reinforces the fact that knowing which category a food falls into does not always offer much predictive power with respect to its nutrient content.

The observed differences among plant types reported here is a new finding for lemur diets, but mirrors previous findings that herbaceous foods in forest clearings used by gorillas provide high-mineral foods (Magliocca & Gautier-Hion, 2002). Previous studies of this sifaka have revealed that hemiparasitic mistletoe (Loranthaceae: *Bakerella clavata*) is a fallback food for CONT groups, being used heavily in the lean season, and additionally has assumed year-round importance in the diet for FRAG groups (Irwin, 2008). This species' food parts (fruit, flowers and leaves) have a unique mineral profile: they are low in Ca (0.14% DM, compared to 0.30 for all foods) and Fe (21.5 ppm, compared to 66.4 for all foods), yet are the best sources of Na (0.21% DM, compared to 0.10 for all foods). More broadly, the importance of non-tree food sources is underscored by the fact that herbs, lianas and epiphytes are some of the richest sources of minerals in the sifaka diet (especially Ca, Mg, Fe, and Cu).

Available data suggest that broader differences among continents and primate radiations may exist (Table VIII). Several studies of colobine monkeys have documented low Na in foods, ranging from 0.0027 to 0.019% DM and gorillas have similarly low values, ranging from 0.0069 to 0.014% (not including Odzala gorilla foods, which are

derived only from clearings and likely a biased sample; Table VIII). Several of these studies posited that certain rare feeding and drinking behaviors (e.g., dead wood and bark consumption, urine and mud puddle drinking, and using herbaceous and swamp plants in clearings) served to increase Na consumption, thus suggesting that regularly-consumed foods are deficient (Cancelliere et al., 2014; Oates, 1978; Rode et al., 2003; Rothman et al., 2006). Na concentrations in sifaka foods were below NRC recommendations (0.096) and lemur diets in general averaged 0.12% (range 0.027–0.22;  $n = 11$ ), but this was higher than haplorhines, which averaged 0.04% (excluding Odzala; range 0.0027–0.18;  $n = 10$ ).

In contrast, Ca concentrations in sifaka foods (average: 0.30% DM) and generally among lemurs (average across 11 studies 0.56) were lower than most colobine monkey and ape diets, as well as two howling monkey populations. However, one clear outlier is *Lemur catta* at Beza Mahafaly (a gallery forest in arid southwestern Madagascar); the others are in eastern rainforests or the high-rainfall Sambirano area of the northwest. If the *Lemur catta* study is omitted, the average for lemurs is 0.32 (range: 0.11–0.76;  $n = 10$ ), a striking contrast to monkeys and apes, which averaged 1.01 (range: 0.35–1.42;  $n = 11$ ). Although more data are needed, this suggests obtaining Ca is difficult for many lemurs, especially in rainforest habitats.

Captive experience corroborates this idea. One study of captive lemurs at the Duke Lemur Center fed a calcium- and vitamin D-enriched diet found serum calcium levels ranging from 9.6 to 12.7 mg/dl, with 3 of 20 subjects "hypercalcemic," or falling higher than the expected "normal" range (Gray et al., 1982). The captive diet was 1.0% Ca DM for the Purina chow and was further enriched with calcium and vitamin D; it's unclear to what degree the hypercalcemia was attributable to the dietary calcium versus the vitamin D (which stimulates the absorption of dietary calcium). Additionally, in 1993 two *Propithecus diadema* at the same colony died shortly after being imported from Mandadia, Madagascar, where low calcium content in

**TABLE III** Linear mixed models using site and season to explain variation in the concentration of selected minerals (percent dry matter) in the diet of diademeds sifakas at Tsinjoarivo, Madagascar

	Final fitted model for percent dry matter				Likelihood ratio tests	
	Fixed effect	Coefficient ± SE	T	p	LR	p
Ca	(Intercept)	0.2668 ± 0.0236	11.30	<0.0001	–	–
	Site CONT	-0.1134 ± 0.0354	-3.20	0.0492	–	–
	Season LEAN	0.0511 ± 0.0118	4.33	<0.0001	–	–
	Site CONT x Season LEAN	0.0447 ± 0.0159	2.82	0.0051	7.51	0.0062
P	(Intercept)	0.1821 ± 0.0023	79.43	<0.0001	–	–
	Site CONT	-0.0198 ± 0.0027	-7.45	0.0050	14.33	0.0002
	Season LEAN	0.0048 ± 0.0023	2.10	0.0366	4.52	0.0336
	Site CONT x Season LEAN	–	–	–	2.44	0.12
Mg	(Intercept)	0.2250 ± 0.0060	37.80	<0.0001	–	–
	Site CONT	0.0162 ± 0.0085	1.92	0.15	–	–
	Season LEAN	-0.0005 ± 0.0049	-0.10	0.92	–	–
	Site CONT x Season LEAN	-0.0159 ± 0.0066	-2.40	0.0168	5.71	0.0169
K	(Intercept)	1.9707 ± 0.0460	42.87	<0.0001	–	–
	Site CONT	-0.6936 ± 0.0616	-11.25	0.0015	–	–
	Season LEAN	0.1204 ± 0.0612	1.97	0.0501	–	–
	Site CONT x Season LEAN	0.3266 ± 0.0830	3.94	0.0001	15.32	0.0001
Na	(Intercept)	0.1057 ± 0.0059	17.77	<0.0001	–	–
	Site CONT	-0.0550 ± 0.0083	-6.63	0.0070	–	–
	Season LEAN	0.0015 ± 0.0058	0.26	0.80	–	–
	Site CONT x Season LEAN	0.0292 ± 0.0079	3.71	0.0002	13.39	0.0003
Fe	(Intercept)	0.0080 ± 0.0007	11.51	<0.0001	–	–
	Site CONT	-0.0006 ± 0.0010	-0.58	0.60	–	–
	Season LEAN	-0.0039 ± 0.0005	-7.82	<0.0001	–	–
	Site CONT x Season LEAN	0.0017 ± 0.0007	2.48	0.0136	6.00	0.0143
Zn	(Intercept)	0.0019 ± 0.0001	22.65	<0.0001	–	–
	Site CONT	-0.0003 ± 0.0001	-2.13	0.12	5.55	0.0185
	Season LEAN	–	–	–	0.02	0.89
	Site CONT x Season LEAN	–	–	–	1.10	0.29
Cu	(Intercept)	0.00066 ± 0.00004	17.43	<0.0001	–	–
	Site CONT	0.00037 ± 0.00005	6.84	0.0064	–	–
	Season LEAN	-0.00010 ± 0.00003	-3.36	0.0009	–	–
	Site CONT x Season LEAN	-0.00017 ± 0.00004	-4.48	<0.0001	19.77	<0.0001
Mn	(Intercept)	0.0238 ± 0.0010	23.75	<0.0001	–	–
	Site CONT	-0.0110 ± 0.0013	-8.20	0.0038	–	–
	Season LEAN	0.0003 ± 0.0014	0.21	0.83	–	–
	Site CONT x Season LEAN	0.0050 ± 0.0019	2.67	0.0079	7.15	0.0075

sifaka foods has been documented (Powzyk, 1997). Necropsies revealed abnormal soft tissue mineralization in the heart, stomach and blood vessels (Duke Lemur Center, unpublished records). Similar pathologies and deaths have been reported for other mammals fed over-enriched diets (e.g., Iberian Lynx (Lopez et al., 2016)). Although circumstantial, this corroborates the idea that enriched captive diets can cause mineral imbalance, particularly in animals accustomed to calcium-poor wild diets, suggesting the existence of highly efficient physiological uptake mechanisms.

## 4.2 | Effects of Seasonality and Habitat Degradation on Mineral Intakes

Previous work on Tsinjoarivo sifakas (Irwin et al., 2014, 2015) revealed that CONT groups exhibit reduced foraging effort and reduced food consumption in the lean season (average 200 g DM) compared to the abundant season (449 g). FRAG groups have lower DM intakes; the reduction is most pronounced in the abundant season, especially for the group occupying the most disturbed



**TABLE IV** Estimated absolute intake of minerals by five diademed sifaka groups at Tsinjoarivo, Madagascar

Absolute intake (g/day)—pooled and by group (CONT1, CONT2/FRAG2, FRAG3, FRAG4)			
	Overall diet	Abundant season	Lean season
Ca	0.57 (0.50, 0.56/0.57, 0.46, 0.69)	0.67 (0.54, 0.71/0.60, –, 0.87)	0.48 (0.46, 0.42/0.55, 0.46, 0.48)
P	0.48 (0.46, 0.62/0.35, 0.25, 0.54)	0.65 (0.61, 0.88/0.40, –, 0.70)	0.32 (0.30, 0.35/0.30, 0.25, 0.36)
Mg	0.64 (0.63, 0.90/0.41, 0.29, 0.67)	0.88 (0.87, 1.27/0.49, –, 0.85)	0.42 (0.38, 0.53/0.34, 0.29, 0.45)
K	4.53 (3.79, 4.71/3.73, 2.67, 6.73)	5.80 (4.43, 6.08/4.19, –, 9.19)	3.35 (3.15, 3.38/3.33, 2.67, 3.89)
Na	0.18 (0.13, 0.18/0.18, 0.19, 0.27)	0.21 (0.15, 0.20/0.22, –, 0.32)	0.15 (0.12, 0.15/0.16, 0.16, 0.20)
Fe	0.0175 (0.0160, 0.0250/0.0131, 0.0058, 0.0169)	0.0266 (0.0237, 0.0369/0.0205, –, 0.0246)	0.0090 (0.0083, 0.0134/0.0068, 0.0058, 0.0080)
Zn	0.0048 (0.0043, 0.0060/0.0034, 0.0028, 0.0058)	0.0065 (0.0058, 0.0084/0.0041, –, 0.0079)	0.0031 (0.0028, 0.0036/0.0028, 0.0028, 0.0035)
Cu	0.0024 (0.0026, 0.0040/0.0012, 0.0007, 0.0019)	0.0036 (0.0039, 0.0058/0.0015, –, 0.0026)	0.0013 (0.0012, 0.0022/0.0009, 0.0007, 0.0012)
Mn	0.045 (0.037, 0.047/0.042, 0.035, 0.059)	0.055 (0.046, 0.061/0.050, –, 0.068)	0.036 (0.029, 0.034/0.035, 0.035, 0.048)

habitat, FRAG2 (lean season: 159 g, abundant season: 223 g). The fact that the site:season interaction in LMMs was usually significant likely reflects this difference in the foraging outcomes for CONT and FRAG groups, as well as differences in species composition of the diet, including FRAG groups' increased reliance on mistletoes (Irwin, 2008).

In the LMMs exploring variation in mineral concentrations in the diet (Table III), the negative coefficient for site revealed that FRAG groups' diets had higher mineral concentration, as predicted. The mixed results for season (positive effect for two minerals, negative effect for two others) suggests that the mineral concentrations in the diet are not particularly higher in one season relative to the other, contrary to predictions; this mirrors a similar finding for macronutrient concentrations in foods (Irwin et al., 2014).

In contrast, the LMMs for absolute intake (Table V) illustrate that season is a significant predictor of all mineral intakes, with lean season intakes much lower than abundant season intakes (following predictions and mirroring the broader trend in DM intakes), but for eight of nine minerals the LMMs did not suggest a meaningful effect of site, contrary to predictions. This suggests that FRAG groups' lower DM intakes and higher mineral concentration in foods roughly offset each other, leaving the FRAG and CONT groups with roughly equal absolute mineral intakes when averaged across seasons.

### 4.3 | Which Foods Are Key Mineral Sources for Sifakas?

Certain unusual feeding and drinking behaviors of wild primates are thought to be driven by a need for supplementing a mineral-poor diet (Cancelliere et al., 2014; Magliocca and Gautier-Hion, 2002; Rode et al., 2003). Sifakas did not consume decaying wood, standing water or urine, and bark was consumed very rarely. They did consume soil (0.30% of feeding time, or 33 s/day), and mineral supplementation may have been one motivation (Krishnamani and Mahaney, 2000; Semel, 2015). Only one food stands out as potentially being selected for mineral content. Young leaves of *Impatiens* sp. were the highest source of Ca (1.57%) and Zn (95 ppm)

and a rich source of P (0.41%) and Fe (174 ppm), but low in macronutrients, being high in fiber (73.0% NDF), average as a protein source (9.8%) and poor in water-soluble carbohydrates (1.9%) (Irwin et al., 2014). Although *Impatiens* leaves only contributed <1% of feeding time (CONT: 0.66%; FRAG: 0.16%), sifakas regularly consumed it in all seasons. This species is low in stature and is found in wet clearings; sifakas come down to the ground to feed, where they are presumably vulnerable to predation.

More broadly, this study revealed that non-tree resources are rich mineral sources, including herbs (*Impatiens* sp.), lianas (e.g., *Zehneria perrieri*), edge trees (*Maesa lanceolata*), and hemiparasites (*Bakerella clavata*). This underscores the importance of dietary diversity, and complementarity of different species and plant types in providing minerals. However, the highest mineral intakes were achieved during the high-fruit season when CONT groups ate fruits of large trees (e.g., *Ocotea*, *Syzygium*, *Garcinia*, *Allophylus*, *Salacia*), which allow high DM, energy and macronutrient intakes (Irwin et al., 2015). These are low or average in mineral concentration, yet the large DM intakes achieved more than compensate for this; thus, one cannot infer that a high-folivory season implies high mineral intakes.

### 4.4 | Are Sifakas Suffering Mineral Deficiencies?

Mineral concentration in sifaka foods (Table I) and daily diets (Table II) at Tsinjoarivo fall below NRC recommendations for six of nine minerals, but there is no evidence that any physiological deficiencies exist. First, during thousands of hours of behavioral observations no outward signs were observed that could be attributed to mineral deficiencies (i.e., no hair loss, weakness, or bone injuries). Second, bloodwork (Irwin et al., 2010) found that serum minerals were largely "normal" (i.e., within reference ranges). For example, twenty-six sifakas sampled showed  $10.16 \pm 0.55$  mg/dl Ca (range 8.9–11.2;  $n = 26$ ; blood was collected during the lean season when intakes would have been lowest), well within the only available reference range for a sifaka (*P. coquerelli*), 8.3–14.4 mg/dl (Teare, 2013).

**TABLE V** Linear mixed models using site and season to explain variation in the daily intake of selected minerals (g) for diademed sifakas at Tsinjoarivo, Madagascar

	Final fitted model for absolute intake (g)				Likelihood ratio tests	
	Fixed effect	Coefficient ± SE	T	p	LR	p
Ca	(Intercept)	0.6716 ± 0.0397	<b>16.91</b>	<0.0001	—	—
	Site CONT	—	—	—	3.53	0.0603
	Season LEAN	-0.1885 ± 0.0381	<b>-4.95</b>	<0.0001	<b>25.01</b>	<0.0001
	Site CONT x Season LEAN	—	—	—	0.16	0.69
P	(Intercept)	0.5315 ± 0.0768	<b>6.92</b>	<0.0001	—	—
	Site CONT	0.2103 ± 0.1107	1.90	0.15	—	—
	Season LEAN	-0.2128 ± 0.0581	<b>-3.67</b>	<b>0.0003</b>	—	—
	Site CONT x Season LEAN	-0.1976 ± 0.0781	<b>-2.53</b>	<b>0.0119</b>	<b>6.20</b>	<b>0.0128</b>
Mg	(Intercept)	0.6464 ± 0.1108	<b>5.84</b>	<0.0001	—	—
	Site CONT	0.4192 ± 0.1619	2.59	0.0811	—	—
	Season LEAN	-0.2673 ± 0.0744	<b>-3.59</b>	<b>0.0004</b>	—	—
	Site CONT x Season LEAN	-0.3402 ± 0.0100	<b>-3.40</b>	<b>0.0007</b>	<b>11.26</b>	<b>0.0008</b>
K	(Intercept)	5.8206 ± 0.6517	<b>8.93</b>	<0.0001	—	—
	Site CONT	—	—	—	0.55	0.46
	Season LEAN	-2.3719 ± 0.5229	<b>-4.54</b>	<0.0001	<b>20.52</b>	<0.0001
	Site CONT x Season LEAN	—	—	—	0.99	0.32
Na	(Intercept)	0.2646 ± 0.0264	<b>10.03</b>	<0.0001	—	—
	Site CONT	-0.0908 ± 0.0382	-2.37	0.0982	—	—
	Season LEAN	-0.0897 ± 0.0191	<b>-4.69</b>	<0.0001	—	—
	Site CONT x Season LEAN	0.0523 ± 0.0257	<b>2.04</b>	<b>0.0425</b>	<b>4.30</b>	<b>0.0381</b>
Fe	(Intercept)	0.0261 ± 0.0023	<b>11.14</b>	<0.0001	—	—
	Site CONT	—	—	—	2.54	0.11
	Season LEAN	-0.0174 ± 0.0015	<b>-11.72</b>	<0.0001	<b>116.65</b>	<0.0001
	Site CONT x Season LEAN	—	—	—	1.83	0.18
Zn	(Intercept)	0.0065 ± 0.0006	<b>11.54</b>	<0.0001	—	—
	Site CONT	—	—	—	0.39	0.53
	Season LEAN	-0.0033 ± 0.0004	<b>-7.71</b>	<0.0001	<b>55.16</b>	<0.0001
	Site CONT x Season LEAN	—	—	—	1.47	0.23
Cu	(Intercept)	0.00196 ± 0.00049	<b>4.01</b>	<b>0.0001</b>	—	—
	Site CONT	0.00286 ± 0.00072	<b>4.00</b>	<b>0.0280</b>	—	—
	Season LEAN	-0.00098 ± 0.00033	<b>-2.92</b>	<b>0.0037</b>	—	—
	Site CONT x Season LEAN	-0.00211 ± 0.00045	<b>-4.71</b>	<0.0001	<b>21.53</b>	<0.0001
Mn	(Intercept)	0.0556 ± 0.0040	<b>13.90</b>	<0.0001	—	—
	Site CONT	—	—	—	1.13	0.29
	Season LEAN	-0.0192 ± 0.0027	<b>-7.17</b>	<0.0001	<b>49.02</b>	<0.0001
	Site CONT x Season LEAN	—	—	—	0.36	0.55

Although sparse, the literature on calcium requirements in primates is a good example of the difficulty inherent in estimating whether intake is adequate. Sifakas' overall dietary concentration is only 30% of the recommended level, yet their mass-specific intake of 116 mg/kg is much higher than the recommended level of 14 mg/kg for humans (Otten, Hellwing, & Meyers, 2006). Looking more directly at experimental studies can yield limited insights. Griffiths, Hunt, Zimmerman, Finberg, and Cuttino (1975) detected osteoporosis in growing female *Macaca mulatta* fed a low-calcium

diet consisting of 200 g of food per day containing 0.3 g Ca (0.15% DM, less than the observed sifaka diet). If all offered food was consumed, this represented a mass-specific intake of 150 mg/kg (higher than sifaka intakes) at the beginning of the study, when monkeys were ~2 kg, and 60 mg/kg (lower than sifaka intakes) at the end, when monkeys were ~5 kg. Garruto et al. (1989) detected motor neuron pathologies in *Macaca fascicularis* maintained on a diet containing 0.32% DM Ca (considerably higher than the sifaka diet) for 3.5 years (food intakes not reported).

**TABLE VI** Dietary intakes scaled to body mass (mg/kg) for key minerals in the diet of sifakas, mountain gorillas, compared to recommendations for humans<sup>a</sup>

	Sifaka intake (overall/abundant/lean)	Gorilla (silverback/female/juvenile) <sup>b</sup>	Recommendation for human male 19–30 yr <sup>c</sup>
Ca	116/135/98	392/733/931	14.3 (AI)
P	98/132/66	67/131/192	8.29 (EAR)
Mg	130/179/85	116/225/292	4.71 (EAR)
K	926/1179/691	612/1013/1597	67.1 (AI)
Na	37/44/31	0.05/0.06/0.08 (1.33/1.94/3.20) <sup>d</sup>	21.4 (AI)
Fe	3.54/5.36/1.84	2.42/4.34/7.52	0.086 (EAR)
Zn	0.97/1.32/0.64	0.64/1.34/2.09	0.134 (EAR)
Cu	0.49/0.72/0.27	0.18/0.33/0.50	0.010 (EAR)
Mn	9.16/11.14/7.33	8.30/16.20/21.50	0.033 (AI)

<sup>a</sup>These comparisons assume an allometry of one, which may not be the best basis for comparison, as sifakas are much smaller than gorillas and humans.

<sup>b</sup>Following (Rothman, Dierenfeld, Hintz, & Pell, 2008) (reported values are mg/kg, not mg per unit of M<sup>0.762</sup> as indicated).

<sup>c</sup>Values calculated from raw daily intakes using reference weight of 70 kg (AI: Adequate Intake; EAR: Estimated Average Requirement) (Otten et al., 2006).

<sup>d</sup>Numbers in parentheses refer to days on which dead wood was consumed

**TABLE VII** Selected mineral ratios (by weight) in the diet of diademed sifakas at Tsinjoarivo, Madagascar

	Overall diet	Abundant season	Lean season	Recommended ratio <sup>a</sup>
Ca:P	1.40	1.18	1.62	1.72
Ca:Na	3.41	3.35	3.47	0.67
Ca:K	0.14	0.13	0.15	0.21
Ca:Mg	1.09	0.87	1.30	3.03
Na:Mg	0.37	0.32	0.42	4.55
Zn:Cu	2.51	2.25	2.75	13.43
Fe:Cu	8.61	10.09	7.23	8.57

<sup>a</sup>Ratios derived from Adequate Intake (AI) and Estimated Average Requirement (EAR) values published for USA males, 19–30 years (Otten et al., 2006).

Thus, assessing whether mineral intake is adequate faces several difficulties. First, it remains unclear whether to focus on proportional or absolute intakes (sifaka intakes would seem adequate by the first measure, but not the second). Our study illustrates how the amount of food consumed can vary drastically across seasons in wild populations, causing a decoupling of proportional and absolute measures of nutrients (i.e., proportional intakes may be highest in one season while absolute intakes are highest in the other). Second, humans may be unusual in their low micronutrient requirements (Milton, 2003). Our study shows the potential for conflicting messages: sifaka diets are below NRC recommended concentrations for primates (for most minerals; Table II), yet absolute intakes per unit body mass are much higher than human dietary recommendations (Table VI). This limits comparisons because the non-human primate literature currently contains only percentage-based recommendations. Third, typical lab analyses do not account for differences in bioavailability (some mineral content in foods may be inaccessible to the consumer). This depends not just on what chemical complexes the minerals are found within, but other components of the diet too; for example, increasing protein and Na intake causes greater Ca excretion, but higher vitamin D intake increases Ca absorption (National Research Council, 2003; Nordin,

2000; Otten et al., 2006). Finally, serum levels can be misleading; for example the Ca balance in the body is buffered against temporary dietary inadequacy by the massive Ca store in bone (Otten et al., 2006).

#### 4.5 | Future Directions

Several limitations make it difficult to draw broader conclusions, and assessing whether observed foods and diets are adequate remains difficult, for the reasons outlined above. Although NRC requirements (National Research Council, 2003) are undoubtedly useful in captive management, they are conservative, as they: (1) take into account that minerals in primate diets may not be bioavailable; (2) aim to provide minerals at levels that avoid upregulation of intestinal absorption; and (3) cover a wide variety of primates of different body masses and digestive systems, and individuals of varying reproductive state. The financial and physiological costs of slightly overshooting mineral targets are low relative to the cost of incurring deficiencies. Additionally, the data on which current NRC recommendations are based are relatively few, and ethical considerations make it unlikely that captive experiments similar to those that contributed most of those data are unlikely to be undertaken in the

**TABLE VIII** Mineral content of food items consumed by sifakas at Tsinjoarivo compared to wild primates at other sites (direct comparisons should be made with caution, as these species differ in digestive function and capabilities and their foods vary in mineral bioavailability)

Population	n	Ca (% DM)	P (% DM)	Mg (% DM)	K (% DM)	Na (% DM)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
<i>Propithecus diadema</i> , Tsinjoarivo (this study)	75	0.30	0.23	0.27	1.85	0.096	66.2	25.0	9.8	275
<i>Propithecus diadema</i> , Tsinjoarivo (Semel, 2015)	9	0.31	0.19	0.25	2.48	0.08	89.8	47.6	11.3	482.4
<i>Eulemur fulvus</i> , Tsinjoarivo (Semel, 2015)	9	0.76	0.14	0.33	2.31	0.03	175.7	66.9	11.4	396
<i>Propithecus diadema</i> , Mantadia (Powzyk, 1997)	16	0.29	0.19	0.32	–	0.16	61.0	27.5	14.9	176
<i>Indri indri</i> , Mantadia (Powzyk, 1997)	13	0.24	0.15	0.30	–	0.22	46.1	16.6	13.1	257
<i>Haplemur alaotrensis</i> , Lac Alaotra (Pollock, 1986) <sup>a</sup>	2	0.15	0.32	0.13	4.05	–	76.5	29.0	–	195
<i>Haplemur alaotrensis</i> , Lac Alaotra (Mutschler, 1999)	14	0.52 <sup>b</sup>	–	–	–	–	–	–	–	–
<i>Eulemur macaco</i> , Ampasikely (Simmen, Bayart, Marez, & Hladik, 2007)	23	0.11	0.15	–	–	–	–	–	–	–
<i>Varecia variegata</i> , Betampona (Schmidt et al., 2010)	105	0.40	0.12	0.20	1.87	0.20	100.4	50.8	10.1	159
<i>Daubentonia madagascariensis</i> , Nosy Mangabe (Sterling, Dierenfeld, Ashbourne, & Feistner, 1994)	4	0.14	0.56	0.32	1.06	0.14	2894	66.5	18.5	53
<i>Lemur catta</i> , Beza Mahafaly (LaFleur & Gould, 2009)	34	2.89	0.22	0.47	3.24	0.027	–	–	–	–
<i>Gorilla beringei</i> , Bwindi (Cancelliere et al., 2014)	84	1.09	0.29	0.38	2.32	0.010	136.8	31.9	7.3	250
<i>Gorilla beringei</i> , Mt. Kahuzi (Casimir, 1975)	8	1.03	–	0.51	2.12	0.0069	–	–	–	–
<i>Gorilla gorilla</i> , Campo (Calvert, 1985)	83	0.83	0.15	0.25	2.88	0.014	152.7	49.2	11.3	325
<i>Gorilla gorilla</i> , Odzala (Magliocca & Gautier-Hion, 2002) <sup>c</sup>	4	1.28	0.80	0.59	3.82	0.79	–	–	–	–
<i>Alouatta palliata</i> , Barro Colorado Island (Nagy & Milton, 1979)	10	1.32	0.21	0.44	2.40	0.18	85.3	–	16.4	62
<i>Alouatta pigra</i> , Monkey River (Behie & Pavelka, 2012)	99	0.98	0.27	0.45	1.78	0.034	98.2	31.1	14.7	–
<i>Alouatta pigra</i> , Community Baboon Sanctuary & Cockscomb Basin Wildlife Sanctuary (Silver et al., 2000)	60	0.75	0.26	0.36	–	0.084	122.2	28.2	12.2	97
<i>Colobus guereza</i> , Kibale (Rode et al., 2003)	– <sup>d</sup>	1.01	–	0.25	1.70	0.019	158.9	33.5	9.7	72.8
<i>Ptilocolobus tephrosceles</i> , Kibale (Rode et al., 2003)	– <sup>d</sup>	1.05	–	0.27	1.71	0.019	149.4	29.1	9.2	68.2
<i>Colobus guereza</i> , Kakamega <sup>e</sup> (Fashing, Dierenfeld, & Mowry, 2007)	22	1.42	0.27	0.32	1.84	0.010	141	26.7	12.9	184
<i>Nasalis larvatus</i> , Tanjung Puting (Yeager et al., 1997)	22	0.35	0.15	0.22	1.28	0.0027	34	17.8	–	56

<sup>a</sup>Only two foods were sampled but this species' diet is extremely monotonous; these two species accounted for 77 of feeding time in a subsequent study (1999).

<sup>b</sup>If "marginal" foods excluded, average drops to 0.30%.

<sup>c</sup>Sampling only included plants consumed in clearings, which were thought to be selected for high mineral content.

<sup>d</sup>Average weighted using proportion of foraging time devoted to each species.

<sup>e</sup>Soil excluded from calculations.

future. Further research is necessary to develop ways of assessing adequacy in more direct ways than simply comparing intakes with published recommendations.

It is also important to recognize the inherent limitations of wild studies. We sampled intake rates using visual observations and used averages from these samples in calculations of mineral intakes. This introduces a potentially large source of error not seen in captive studies (where it's often possible to directly weigh food offered and subtract the mass of food not eaten (Nagy and Milton, 1979)); more work is needed to understand the magnitude of this error, and to minimize it in future studies.

One key future direction is assessing the bioavailability of nutrients. Minerals may be more or less accessible for uptake depending on the molecules in which they are found, and the balance of other ions in the gut lumen. Analyses comparing mineral

content in primates' foods and feces would be a useful technique in quantifying bioavailability (Cancelliere et al., 2014; Nagy and Milton, 1979). Additionally, primate species and radiations likely differ in their physiological potential for absorption. For example, the possibility that Ca is a particular challenge for lemurs and caused an evolved physiological response is suggested by the fact that: (1) lemur diets tend to be Ca-poor, particularly in rainforests, and (2) captive sifakas maintained on enriched diet have exhibited abnormal mineralized deposits. Our understanding of the causes and consequences of this interspecific variation could be improved through a combination of field studies that quantify the mineral content of wild diets across species and habitats, captive studies examining absorption rates, and studies in either context examining food selection. A second key future direction is how interactions among nutrients affects absorption; this includes other minerals

(Table VII) as well as other interactions with vitamins, macronutrients and fiber.

Advances in this field will be important for both captive and wild populations. Although in captivity there may be only minor consequences when minerals are overfed, the death of the *Propithecus diadema* previously mentioned at the Duke Lemur Center is an illustrative example: although cause of death was not directly linked to mineral imbalances (Duke Lemur Center, unpublished records), the possibility that a diet can be “too enriched” is concerning. In the wild, as habitat change progresses, nutritional impacts will be one of the many challenges faced by wild populations. Understanding the role of dietary diversity and complementary in both macronutrient and micronutrient inputs will be crucial for managers seeking to mitigate ecological pressures arising from the changing composition of the habitat.

## ACKNOWLEDGMENTS

We thank the Government of Madagascar, CAFF/CORE and the Ministry of Environment, Ecology, Oceans and Forests for research authorization. For research facilitation we thank P. Wright, B. Andriamihaja, the Malagasy Institute for the Conservation of Tropical Environments (MICET), and the Institute for the Conservation of Tropical Environments (ICTE). For data and sample collection assistance, we thank Maitsoanala research assistants. We thank C. Walsh, M. Van Amburgh, D. Ross, and the Cornell University Animal Nutrition Laboratory for analysis help, Dr. K. Glander and Dr. N. Barber for discussions, and Dr. C. Williams and Dr. A. Yoder for allowing access to Duke Lemur Center records. We thank K. Samonds and two anonymous reviewers for comments on an earlier draft.

## REFERENCES

- Barboza P. S., Parker K. L., & Hume I. D. (2009). *Integrative wildlife nutrition* (p. 342). Berlin: Springer.
- Behie, A. M., & Pavelka, M. S. M. (2012). The role of minerals in food selection in a black howler monkey (*Alouatta pigra*) population in Belize following a major hurricane. *American Journal of Primatology*, *74*, 1054–1063.
- Calvert, J. J. (1985). Food selection by western gorillas (*G.g. gorilla*) in relation to food chemistry. *Oecologia*, *65*, 236–246.
- Cancelliere, E. C., DeAngelis, N., Nkurunungi, J. B., Raubenheimer, D., & Rothman, J. M. (2014). Minerals in the foods eaten by Mountain Gorillas (*Gorilla beringei*). *PLoS ONE*, *9*, 10.
- Casimir, M. J. (1975). Feeding ecology and nutrition of an eastern gorilla group in the Mt. Kahuzi region (République du Zaïre). *Folia Primatologica*, *24*, 81–136.
- Chaves, O. M., Stoner, K. E., Angeles-Campos, S., & Arroyo-Rodriguez, V. (2011). Wood consumption by Geoffroy's spider monkeys and its role in mineral supplementation. *PLoS ONE*, *6*.
- Fashing, P. J., Dierenfeld, E. S., & Mowry, C. B. (2007). Influence of plant and soil chemistry on food selection, ranging patterns, and biomass of *Colobus guereza* in Kakamega Forest. *Kenya. International Journal of Primatology*, *28*, 673–703.
- Felton, A. M., Felton, A., Lindenmayer, D. B., & Foley, W. J. (2009). Nutritional goals of wild primates. *Functional Ecology*, *23*, 70–78.
- Garruto, R. M., Shankar, S. K., Yanagihara, R., Salazar, A. M., Amyx, H. L., Gajdusek, D. C. (1989). Low-calcium, high-aluminum diet-induced motor neuron pathology in cynomolgus monkeys. *Acta Neuropathologica*, *78*, 210–219.
- Gray, T. K., Lester, G. E., Moore, G., Crews, D., Simons, E. L., Stuart, M. (1982). Serum concentrations of calcium and vitamin D metabolites in prosimians. *Journal of Medical Primatology*, *11*, 85–90.
- Griffiths, H. J., Hunt, R. D., Zimmerman, R. E., Finberg, H., & Cuttino, J. (1975). The role of calcium and fluoride in osteoporosis in rhesus monkeys. *Investigative Radiology*, *10*, 263–268.
- Irwin M. T. (2006). *Ecological impacts of forest fragmentation on diademedsifakas (Propithecus diadema) at Tsinjoarivo, eastern Madagascar: Implications for conservation in fragmented landscapes [Ph.D.]* (p. 410). Stony Brook, NY: Stony Brook University.
- Irwin, M. T. (2008). Feeding ecology of diademedsifakas (*Propithecus diadema*) in forest fragments and continuous forest. *International Journal of Primatology*, *29*, 95–115.
- Irwin, M. T., Junge, R. E., Raharison, J. L., & Samonds, K. E. (2010). Variation in physiological health of diademedsifakas across intact and fragmented forest at Tsinjoarivo, eastern Madagascar. *American Journal of Primatology*, *72*, 1013–1025.
- Irwin M. T., & Raharison F. J.-L. (in press). Interpreting small-scale patterns of ranging by primates: What does it mean, and why does it matter? In C. A. Shaffer, F. L. Dolins, J. R. Hickey, N. P. Nibbelink, & L. M. Porter (Eds.), *GPS and GIS for primatologists: A practical guide to spatial analysis*. Cambridge: Cambridge University Press.
- Irwin, M. T., Raharison, J.-L., Raubenheimer, D., Chapman, C. A., & Rothman, J. M. (2014). Nutritional correlates of the “lean season”: Effects of seasonality and frugivory on the nutritional ecology of diademedsifakas. *American Journal of Physical Anthropology*, *153*, 78–91.
- Irwin, M. T., Raharison, J.-L., Raubenheimer, D. R., Chapman, C. A., & Rothman, J. M. (2015). The nutritional geometry of resource scarcity: Effects of lean seasons and habitat disturbance on nutrient intakes and balancing in wild sifakas. *PLoS ONE*, *10*, e0128046.
- Janson C. H., & Chapman C. A. (1999). *Resources and primate community structure. Primate Communities* (pp. 237–267). Cambridge: Cambridge University Press.
- Krishnamani, R., & Mahaney, W. C. (2000). Geophagy among primates: Adaptive significance and ecological consequences. *Animal Behaviour*, *59*, 899–915.
- LaFleur, M., & Gould, L. (2009). Feeding outside the forest: The importance of crop raiding and an invasive weed in the diet of gallery forest ring-tailed lemurs following a cyclone at the Beza Mahafaly Special Reserve, Madagascar. *Folia Primatologica*, *80*, 233–246.
- Lambert J. E., (2011). Primate nutritional ecology: Feeding biology and diet at ecological and evolutionary scales. In C. J. Campbell, A. Fuentes, K. C. MacKinnon, S. K. Bearder, & R. M. Stumpf (Eds.), *Primates in perspective* (pp. 512–522). New York: Oxford University Press.
- Lopez, I., Pineda, C., Muñoz, L., Raya, A., Lopez, G., Aguilera-Tejero, E. (2016). Chronic vitamin D intoxication in captive iberian lynx (*Lynx pardinus*). *PLoS ONE*, *11*, e0156331.
- Magliocca, F., & Gautier-Hion, A. (2002). Mineral content as a basis for food selection by western lowland gorillas in a forest clearing. *American Journal of Primatology*, *57*, 67–77.
- Milton, K. (2003). Micronutrient intakes of wild primates: are humans different? *Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology*, *136*, 47–59.
- Mutschler T., (1999). Folivory in a small-bodied lemur: The nutrition of the Alaotran gentle lemur (*Haplemur griseus alaotrensis*). In B. Rakotosamimanana, H. Rasamimanana, J. U. Ganzhorn, & S. M. Goodman (Eds.), *New directions in lemur studies* (pp. 221–239). New York: Plenum.

- Nagy, K. A., & Milton, K. (1979). Aspects of dietary quality, nutrient assimilation and water balance in wild howler monkeys (*Alouatta palliata*). *Oecologia*, 39, 249–258.
- National Research Council. (2003). *Nutrient requirements of nonhuman primates* (p. 286). Washington, DC: National Academies Press.
- Nordin, B. E. C. (2000). Calcium requirement is a sliding scale. *American Journal of Clinical Nutrition*, 71, 1381–1383.
- Oates, J. F. (1978). Water-plant and soil consumption by guereza monkeys (*Colobus guereza*): A relationship with minerals and toxins in the diet?. *Biotropica*, 10, 241–253.
- Otten J. J., Hellwing J. P., & Meyers L. D. (2006). *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*. Washington, DC: The National Academies Press.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & Team, R. C. (2015). NLME: Linear and non-linear mixed effects models. R Package version 3.1-122.
- Pollock, J. (1986). A note on the ecology and behavior of *Haplemur griseus*. *Primate Conservation*, 7, 97–101.
- Powzyk J. A. (1997). *The socio-ecology of two sympatric indriids: Propithecus diadema diadema and Indri indri, a comparison of feeding strategies and their possible repercussions on species-specific behaviors [PhD]* (p. 307). Durham, NC: Duke University.
- R Core Team. (2015). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rode, K. D., Chapman, C. A., Chapman, L. J., & McDowell, L. R. (2003). Mineral resource availability and consumption by *Colobus* in Kibale national park, Uganda. *International Journal of Primatology*, 24, 541–573.
- Rode, K. D., Chapman, C. A., McDowell, L. R., & Stickler, C. (2006). Nutritional correlates of population density across habitats and logging intensities in redtail monkeys (*Cercopithecus ascanius*). *Biotropica*, 38, 625–634.
- Rothman, J. M., Dierenfeld, E. S., Hintz, H. F., & Pell, A. N. (2008). Nutritional quality of gorilla diets: Consequences of age, sex, and season. *Oecologia*, 155, 111–122.
- Rothman, J. M., Van Soest, P. J., & Pell, A. N. (2006). Decaying wood is a sodium source for mountain gorillas. *Biology Letters*, 2, 321–324.
- Schmidt, D. A., Iambana, R. B., Britt, A., Junge, R. E., Welch, C. R., Porton, I. J., Kerley, M. S. (2010). Nutrient composition of plants consumed by Black and White Ruffed Lemurs, *Varecia variegata*, in the Betampona Natural Reserve. *Madagascar. Zoo Biology*, 29, 375–396.
- Semel B. P. 2015. A multi-species approach to elucidating the ecological function of geophagy [MA thesis]: Northern Illinois University. 94 p.
- Silver, S. C., Ostro, L. E. T., Yeager, C. P., & Dierenfeld, E. S. (2000). Phytochemical and mineral components of foods consumed by black howler monkeys (*Alouatta pigra*) at two sites in Belize. *Zoo Biology*, 19, 95–109.
- Simmen, B., Bayart, F., Marez, A., & Hladik, A. (2007). Diet, nutritional ecology, and birth season of *Eulemur macaco* in an anthropogenic forest in Madagascar. *International Journal of Primatology*, 28, 1253–1266.
- Simpson S. J., & Raubenheimer D. (2012). *The Nature of Nutrition: An integrative framework from animal adaptation to human obesity* (p. 248). Princeton: Princeton University Press.
- Sterling, E. J., Dierenfeld, E. S., Ashbourne, C. J., & Feistner, A. T. C. (1994). Dietary intake, food composition and nutrient intake in wild and captive populations of *Daubentonia madagascariensis*. *Folia Primatologica*, 62, 115–124.
- Teare J. A. 2013. Propithecus\_coquereli\_No\_selection\_by\_gender\_All\_ages\_combined\_Conventional\_American\_units\_2013\_C D.html. In: ISIS Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource, International Species Information System, Bloomington, MN.
- Yeager, C. P., Silver, S. C., & Dierenfeld, E. S. (1997). Mineral and phytochemical influences on foliage selection by the proboscis monkey (*Nasalis larvatus*). *American Journal of Primatology*, 41, 117–128.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Irwin MT, Raharison J-L, Chapman CA, Junge RE, Rothman JM. Minerals in the foods and diet of diademed sifakas: Are they nutritional challenges? *Am J Primatol*. 2017;79:e22623. <https://doi.org/10.1002/ajp.22623>