Received: 28 July 2022 | Revised: 5 December 2022 | Accepted: 18 February 2023

DOI: 10.1002/ajp.23484

RESEARCH ARTICLE

AMERICAN JOURNAL OF PRIMATOLOGY WILEY

Nutrient balancing in a fruit-specialist primate, the black-and-white ruffed lemur (*Varecia variegata*)

Nina Beeby[1,2] | Jessica M. Rothman[1,2,3] | Andrea L. Baden[1,2,3]

[1]Department of Anthropology, The Graduate Center of City University of New York, New York, USA

[2]The New York Consortium in Evolutionary Primatology (NYCEP), New York, USA

[3]Department of Anthropology, Hunter College of City University of New York, New York, USA

Correspondence
Nina Beeby, Department of Anthropology, The Graduate Center of CUNY, 365 Fifth Ave, New York, NY 10016, USA.
Email: nbeeby@gradcenter.cuny.edu

Funding information
Graduate Center; Hunter College; PSC-CUNY; Leakey Foundation

Abstract

Animals' foraging behavior and dietary choices are, in part, driven by their ultimate function: to meet nutritional demands. However, depending on their degree of dietary specialization and the availability and distribution of food resources in their environment, species may utilize different nutritional strategies. With shifting plant phenology, increasing unpredictability of fruiting, and declining food quality in response to anthropogenic climate change, existing nutritional constraints may become exacerbated. Such changes are especially concerning for Madagascar's endemic fruit specialists given the nutrient-limitation of the island's landscapes. In this study, we examined the nutritional strategy of one such fruit-specialist primate, the black-and-white ruffed lemur (*Varecia variegata*), over a 12-month period (January to December 2018) in Ranomafana National Park, Madagascar. We hypothesized that *Varecia* would balance nonprotein energy (NPE) to protein (AP) at a high ratio similar to other frugivorous primates, and that they would prioritize protein intake given their high degree of frugivory. We found that *Varecia* balance NPE:AP at a ratio of 11:1, higher than in any other primate studied to date; however, diets shifted such that nutrient balancing varied seasonally (12.6:1 abundant–9.6:1 lean). *Varecia* meet NRC suggested recommendations of 5–8% of calories from protein, despite having a diet mostly comprising fruits. However, seasonal shifts in NPE intakes result in significant energy shortfalls during fruit-lean seasons. Flowers provide an important source of NPE during these periods, with flower consumption best predicting lipid intake, suggesting this species' ability to shift resource use. Nevertheless, achieving adequate and balanced nutrient intakes may become precarious in the face of increasing unpredictability in plant phenology and other environmental stochasticities resulting from climate change.

KEYWORDS
carbohydrate, lipid, Madagascar, nutritional geometry, protein

---

Abbreviations: AP, available protein; NPE, nonprotein energy; NRC, National Research Council.

https://doi.org/10.1002/ajp.23484

1 | INTRODUCTION

The ultimate function underlying many animal foraging behaviors is to consume adequate nutrients (Barboza et al., 2009). As such, characterizing nutrition is key to understanding the selective forces driving species' foraging strategies and ultimately how individuals are able to persist in their environment (DeGabriel et al., 2014; Janson & Chapman, 1999; McNab, 2002; Robbins, 2012). Adequate nutrition is a prerequisite for survival and reproduction and there are myriad pressures influencing how an individual can procure the macro- and micro-nutrients it needs. Nutritional ecology aims to understand such pressures and the relationships among nutrition, diet composition, food selection, behavior, and habitat use. These relationships are complex, as requirements vary according to individual circumstance, including factors such as life history stage and health (Raubenheimer & Simpson, 2016). Animal foraging is often adjusted to meet specific nutritional goals based on fluctuating food availability, distribution, and nutrient contents of foods (Lambert & Rothman, 2015). Several models have been used to better understand animal nutritional strategies and potential nutrient limitations, focusing on different dietary and nutritional parameters (Belovsky, 1984; Felton, Felton, Lindenmayer, et al., 2009; MacArthur & Pianka, 1966; Pyke, 1984; Stephens & Krebs, 2019). More recent focus has been on nutrient balancing (Raubenheimer & Simpson, 2004) whereby species regulate intakes of multiple nutrients simultaneously (Simpson et al., 2003) and achieve a balanced intake of nutrients according to their physiological requirements.

Among vertebrates, strict frugivory—that is, 100% fruit contribution to the diet—as a dietary strategy is rare, even during seasonal fruit abundance (Delorme & Thomas, 1999; Izhaki & Safriel, 1989; Terborgh, 1986; Thomas, 1984). Fruits are distributed unevenly in space and time (Brockman & Van Schaik, 2005) and, although easy to digest, are too low in protein to meet species' total metabolic requirements (Donati et al., 2017; Izhaki & Safriel, 1989; Lambert et al., 2014). As such, most frugivorous birds and mammals supplement their diets with protein-rich foods, such as leaves or insects, to satisfy their protein needs (e.g., Galetti et al., 2001; G. L. Herrera et al., 2002; M. L. G. Herrera et al., 2009; Karr et al., 1990; Oftedal et al., 1996; Rode & Robbins, 2000). Nevertheless, fruit specialists (i.e., those that are anatomically or physiologically constrained to frugivory) can still be susceptible to nutrient limitations if their adaptations hinder their ability to extract nutrients from less digestible foods (Lambert, 1998; Saldaña-Vázquez et al., 2015).

Primates are among the world's frugivorous mammals, and even those which are not classified as frugivores often consume fruits when they are available (Hemingway & Bynum, 2005). To satisfy their physiological needs, primates prioritize nonprotein energy (NPE) (Rothman et al., 2011) or available protein (AP) (Dröscher et al., 2016; Felton, Felton, Raubenheimer, et al., 2009; Takahashi et al., 2021), while still others balance NPE and AP across a range of ratios (Z. W. Cui et al., 2018; Dunham & Rodriguez-Saona, 2018; Johnson et al., 2017). Of these strategies, frugivorous primates generally tend to prioritize AP while maximizing NPE intake within that constraint (e.g., Uwimbabazi et al., 2021). More generalist frugivores seem able to engage in flexible behavioral and nutritional tactics to overcome dietary constraints (Cui et al., 2019; Takahashi et al., 2021). By contrast, specialist frugivores may have more limited ability to alter their diet in response to changing food availability and therefore may be particularly challenged to acquire sufficient nutrients and energy under changing ecological conditions.

Of the world's primate habitats, Madagascar's eastern rainforests exhibit marked seasonality in rainfall, temperature, and fruit availability (e.g., Dunham et al., 2018; Hemingway, 1996; Razafindratsima & Dunham, 2016; Wright, 2006). Compared to other tropical regions, Madagascar's climate is often described as harsh, suffering frequent stochastic events such as droughts and cyclones (Dewar & Richard, 2007; Gould et al., 1999; Lewis & Bannar-Martin, 2012; Wright, 1999; but see Federman et al., 2017). Moreover, Madagascar's plant communities are characterized by smaller, slower growing trees, lower fruit abundance and quality, lower soil fertility, and greater interannual variation in fruiting periodicity than those in other tropical regions (Dewar & Richard, 2007; Donati et al., 2017). In fact, Madagascar's rainforests can experience up to 6 months of the year with significantly reduced fruit availability (Dewar & Richard, 2007; Dunham et al., 2018; Wright et al., 2005). Further exacerbating these environmental selection pressures, low nitrogen concentrations in Malagasy fruits means that protein requirements for frugivores may be especially difficult to satisfy (Donati et al., 2017; Ganzhorn et al., 2009)—effects which are likely to become more pronounced with increasing habitat modification (Vieilledent et al., 2018) and climate change (Brown & Yoder, 2015; Dunham et al., 2018; Renner & Zohner, 2018; Rothman et al., 2015). Here, we investigated the nutritional strategy of wild black-and-white ruffed lemurs (*Varecia variegata*), one of Madagascar's few fruit-specialist primates.

Ruffed lemurs (Genus *Varecia*), found in Madagascar's eastern rainforests, are among the largest and most frugivorous of the living lemurs (Baden et al., 2008; Dew & Wright, 1998; Martinez & Razafindratsima, 2014; Razafindratsima et al., 2014; Vasey, 2006; Wright et al., 2011). Although they prefer fruits, they seasonally consume large quantities of flowers and fall back on leaves when other foods are not available (Balko, 1998; Beeby & Baden, 2021; Britt, 2000; Holmes et al., 2016). Despite their dietary breadth, ruffed lemurs exhibit several fruit-specialist traits (Campbell et al., 2000; Edwards, 1995; Edwards & Ulrey, 1999), and have been shown to be highly susceptible to the effects of habitat degradation (White et al., 1995) and climate change (Morelli et al., 2020). Several studies have shown *Varecia* to alter their feeding behavior in response to changes in fruit availability resulting from both climatic seasonality and habitat disturbance (Balko, 1998; Balko & Underwood, 2005; Beeby & Baden, 2021; Britt, 2000; Holmes et al., 2016; Ratsimbazafy, 2002, 2006). Though one study examined the nutrient composition of dietary foods in one *V. variegata* population in eastern Madagascar (Schmidt et al., 2010), to date, there has been no exploration of nutrient intakes in this taxon. Insights into ruffed lemur's nutritional ecology will be crucial to understanding how these fruit specialists are able to cope with the unique challenges of frugivory in Madagascar's eastern forests, and how this might shift in response to anthropogenic climate change.

In this study, we combined observations of feeding behavior with laboratory analyses of macronutrient content of foods to test four hypotheses about the nutritional strategy of black-and-white ruffed lemurs (*V. variegata*). First, based on their highly frugivorous diet (Balko, 1998; Balko & Underwood, 2005; Balko, 1998; Beeby & Baden, 2021; Britt, 2000; Holmes et al., 2016; Ratsimbazafy, 2002, 2006) and preliminary description of the nutrient content of ruffed lemur foods (Schmidt et al., 2010), we hypothesized that (H1) *Varecia* would consume a diet low in protein and high in carbohydrates and lipids, with major food types differing significantly in macronutrient proportions. Second, as they are considered fruit-specialists, we hypothesized that (H2) *Varecia* would also balance the daily intake of NPE and protein at a higher ratio than previously recorded in other primates (Dunham & Rodriguez-Saona, 2018; Felton, Felton, Lindenmayer, et al., 2009; Irwin et al., 2015; Johnson et al., 2013; Martínez-Mota et al., 2016; Rothman et al., 2011; Takahashi et al., 2021; Uwimbabazi et al., 2021). Moreover, given the limited nitrogen available in Malagasy fruits (Donati et al., 2017; Ganzhorn et al., 2009), we hypothesized that (H3) protein would be limited in the *Varecia* diet, meaning protein (AP) intake would be tightly regulated. Resultingly, NPE intakes would likely show marked variation. This rule of compromise known as protein prioritization has been observed in several other frugivorous primates (*Ateles chamek*: Felton, Felton, Lindenmayer, et al., 2009; Felton, Felton, Raubenheimer, et al., 2009; *Cercopithecus mitis*: Takahashi et al., 2021; *Pan troglodytes*: Uwimbabazi et al., 2021). Finally, because resource availability in Madagascar is highly variable, especially so for fruits (Dewar & Richard, 2007; Dunham et al., 2018; Wright et al., 2005), the foods available for *Varecia* to consume and therefore the proportion of food types in the diet are likely to change through time. Therefore, we hypothesized that (H4) daily NPE:AP ratio and macronutrient (NPE, AP, total nonstructural carbohydrates [TNC], lipid) intakes would vary as a function of the relative proportions of fruit, flowers, and leaves in the *Varecia* diet, resulting in *Varecia* experiencing significant seasonal variability in macronutrient intakes.

2 | METHODS

2.1 | Study site and subjects

We collected data on two adjacent communities of black-and-white ruffed lemurs (*V. variegata*) in Ranomafana National Park, Madagascar (RNP; Figure 1), over a 12-month period from January to December 2018. Mangevo [21°22′60″ S, 47°28′0″ E], is a mid-elevation primary rainforest site in the southeast of RNP (Wright et al., 2012). Study subjects comprised $n = 13$ females and $n = 14$ males with radio-collars and $n = 2$ individuals (one female, one male) with collar-tags but no radio-collars.

2.2 | Data collection

2.2.1 | Observational data

Each month we targeted approximately 15 individuals for focal animal follows from 6 a.m. to 6 p.m. Efforts were made to sample individuals evenly by selecting a new focal animal each day from a randomized-ordered list each month. This typically comprised one follow per radio-collared individual per day, plus opportunistic follows on collar-tagged individuals if located before 10 a.m. The total number of hours of focal animal follows was 1383. During focal animal follows one observer collected continuous feeding behavior data for nutrition (Altmann, 1974). This observer recorded feeding bouts on an all-occurrence basis, recording bout duration, species, and part of all plant foods, how individuals processed foods (e.g., discarded skins or seeds), and quantities eaten using standard units (e.g., one fruit, three leaves). Feeding bouts were stopped when the focal animal stopped feeding (extraction, ingestion, chewing, or swallowing) for a period of 30 s or more. Team members reassessed unit measures at regular intervals to ensure consistency. If units could not be accurately counted, a "feeding out of sight" state was recorded to enable accurate calculation of total feeding time. We calculated the proportion of time spent feeding on fruits, leaves, and flowers using the following formula: (plant part feeding time/total feeding time × 100). Alongside continuous observations of each daily focal animal, a second observer from each team marked and GPS mapped feeding trees for later plant sample collection.

2.2.2 | Plant sample collection

We collected plant samples from the 10 most frequently fed on plant taxa (identified by those taxa with the greatest tally of feeding bouts and/or total feeding time that month) on the final 2 days of each month ($n = 68$). Plant samples were collected from plants in which individuals were observed feeding. When this was not possible, we collected samples from conspecific plants at a similar stage of maturity and phenophase, within the home range of the focal individual observed feeding on that taxon. The plant parts sampled included fruit, leaves, flowers, petiole, and one opportunistically sampled gall. After collection, we processed the food items in the same way as the lemurs did (e.g., removing fruit skin or seeds; Rothman et al., 2012). Then, using the same standard units from focal animal follows, we weighed samples to obtain a mean wet weight per unit before drying samples in an Excalibur 6-tray food dehydrator at 50°C for 1–5 days. Once dried, samples were stored in Whirlpak bags at room temperature and out of sunlight.

2.3 | Data analyses

2.3.1 | Nutritional analyses

We milled each sample through a Wiley Mill® with a 1-mm screen before sequentially analyzing macronutrient components as per guidelines established by Rothman et al. (2012). To calculate true dry matter (nonmoisture portion of food matter), samples were dried at 105°C for 16 h to account for adsorbed moisture and reweighed. For ash, samples were burned at 500–550°C. For crude protein, nitrogen content was measured using a Leco TruSpec Nitrogen Analyzer, then multiplied by 6.25 (conversion value) for approximate protein content. A more accurate measure of AP was also calculated using the same combustion technique

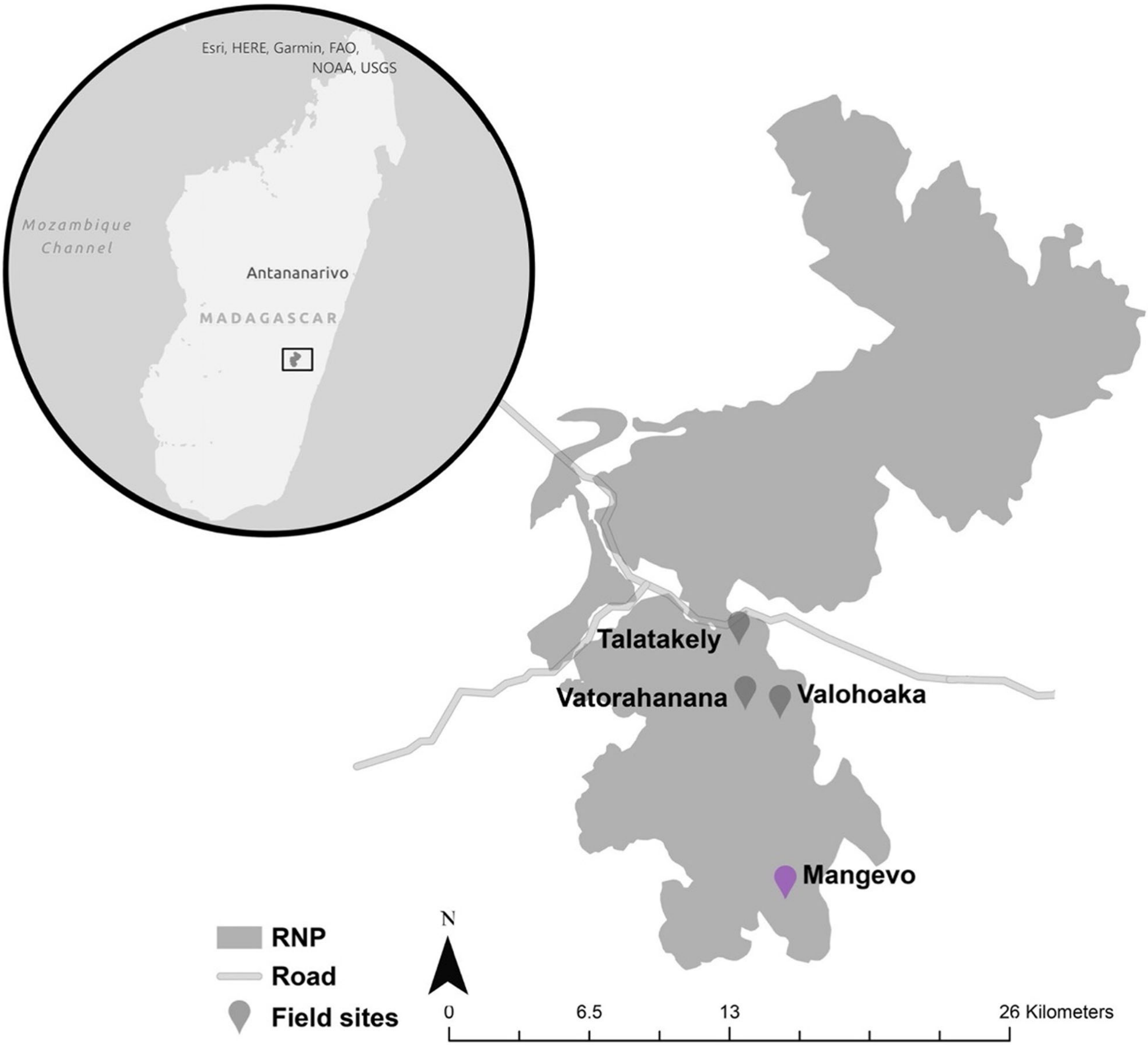

FIGURE 1 Map showing Ranomafana National Park (RNP), located in southeastern Madagascar. The inset map shows the field sites within RNP, including Mangevo, the site of this study (adapted from Baden et al., 2021; Beeby & Baden, 2021).

correcting for insoluble protein. AP was then calculated using post-acid detergent fiber (ADF) samples: (post-ADF N × ADF/100) × 6.25 (Rothman et al., 2012). Ether extract was determined using an ANKOM Fat Analyzer and fibers using an ANKOM Fiber Analyzer (Mertens, 2002; Van Soest et al., 1991).

2.3.2 | Food and nutrient intake calculations

We combined macronutrient contents of each food item with food intakes from feeding observations to determine nutrient intakes. Based on the expected time these animals could spend feeding, we scaled up values from our average 8 h observation days to 13 h days to represent the potential total feeding time. We calculated wet weight ingested per minute for each food item using wet weight × units/min, then translated this to feeding rate using the following formula for each food item: feeding rate = wet weight ingested per minute × (dry unit weight/wet unit weight × 100). Daily dry food ingested (g/day) was calculated as DM (g) = Σ (time spent feeding on given food item per day [min] × average feeding rate for given item [g/min]). From this, we calculated daily nutrient intake (DI) (g/day) using DI = Σ (amount of dry food ingested for given food item × mean nutrient contents of given food item). Each macronutrient was then converted into calories using standard physiological values of 4 kcal/g carbohydrates, 4 kcal/g crude protein, 9 kcal/g lipid, and 3 kcal/g NDF (Conklin-Brittain et al., 2006). The caloric value of NDF was adjusted using a fiber digestibility coefficient of 0.255, determined by Edwards and Ulrey (1999). Finally, calorie values were used to calculate daily metabolizable energy consumed using ME (kcal) = TNC (kcal) + AP (kcal) + Lipid-1 (kcal) + NDF (kcal), daily nonprotein energy as NPE = Lipid + TNC + NDF, and available protein as AP = Σ AP (per day). In cases of missing data, such as periods of feeding out of sight or feeding on nonsampled foods, we used mean nutrient values by plant part (14% of intake values).

2.3.3 | Ecological analyses

Broad seasons (abundant: November to April, and lean: May to October) were assigned using classifications by Beeby & Baden (2021). These were assigned using mean daily temperature (°C) and monthly rainfall (mm) combined with monthly percentage fruit availability as estimated from 585 trees and lianas found within 12 botanical plots (each $50\,\mathrm{m}^2$) established evenly throughout the communities' home ranges (Baden, 2011; Baden et al., 2016).

2.3.4 | Statistical analyses

We used one-way analysis of variances and post hoc Tukey tests to determine whether macronutrient proportions as well as metabolizable energy of major food types (i.e., fruits, flowers, leaves) differ significantly. We then used the geometric framework (Raubenheimer et al., 2009), a modelling approach in which each axis represents a different nutritional component, to examine patterns of macronutrient intake. We constructed two-dimensional model with AP intakes (kcal) on the *x*-axis and NPE intakes (kcal) on the *y*-axis. Each point shows the end-point intakes of NPE and AP on a given day, indicating the ratio of NPE:AP consumed. To statistically examine protein prioritization, we fitted a power function to the relationship between intake and proportion of protein in the diet, based on the presumption that to maintain a constant protein intake (P), on a diet comprising a given proportion of protein (p), Pp^L grams of total food must be eaten (whereby an exponent of $L = -1$ would indicate complete protein priotization: Raubenheimer & Simpson, 2019; Takahashi et al., 2021). We also constructed right-angle mixture triangles (Raubenheimer, 2011), with the proportion of ME intake from protein (AP) on the *x*-axis, carbohydrates (NDF + TNC) on the *y*-axis, and lipids (crude fat) on the implicit axis, to examine relative contributions of each macronutrient to the daily diet. Points represent relative proportions of each nutrient in the *Varecia* diet on a given day. We excluded all days during which the focal animal was observed for fewer than 8 h or was out of sight for more than a combined total of 30 min. Finally, we used linear mixed models (LMMs) to examine whether NPE:AP ratio and intakes of AP, NPE, TNC, lipids, NDF, and mass of food ingested (g) were influenced by (1) season and (2) frugivory in the daily diet. For all LMMs, we controlled for individual ID due to repeated sampling of individuals, and sex in case of sex-differences due to female dominance. We did not control for rank as rank effects are expected to be minimal, given low competition in this species due to individuals spending nearly 50% of their time alone (Baden et al., 2016). Moreover, it was not necessary to control for female reproductive state because data included in this study were from a nonreproductive year for this population.

3 | RESULTS

Overall, foods consumed by *Varecia* were moderate in AP compared to NRC recommendations for nonhuman primates (Table 1; National Research Council, 2003). Fruits were lower in protein contents than other foods, but contrary to our expectation, fruits were not significantly lower in protein than leaves in terms of crude ($p = 0.015$) or AP ($p = 0.213$). Foods were generally moderate in TNC and high in fiber content (NDF, ADF, ADL) compared to published recommendations (National Research Council, 2003; Oftedal, 1991). Lipid contents were generally low; however, some flowers were extremely high in lipids (see Supporting Information: Table S1), likely driving the significant differences in lipid content across food types. Significant differences in macronutrient contents across food types were also seen in total nonstructural carbohydrates (Table 2).

Overall, *Varecia* consumed a mean of 861 g of food per day on a wet weight basis (range: 40–2438 g, SD: 407) and 172 g of food per day on a dry matter basis (range: 10–813 g, SD: 97). Daily calorie intakes showed substantial variation between 21 and 1292 kcal per

TABLE 1 One-way ANOVA results and mean macronutrient concentrations (reported on a % dry matter basis) of $n = 68$ food items in the *V. variegata* diet, collected January–December 2018.

Variable	*F*	*df*	*Pr (>F)*	Means Fruit	Flower	Leaf	Recommended*
Lipid	3.063	3, 63	**0.034**	5.6	9.0	3.6	*NA*
CP	3.535	3, 62	0.020	9.3	11.3	14.6	7–10
AP	1.347	3, 54	0.269	5.9	7.2	9.7	6.4–8
NDF	0.302	3, 62	0.824	48.8	47.4	52.4	10–30
ADF	0.149	3, 62	0.930	40.6	41.1	42.7	5–15
ADL	1.318	3, 62	0.277	21.2	23.2	23.5	*NA*
TNC	2.785	3, 62	**0.048**	30.3	25.4	20.2	*NA*

Note: Significant results are in bold. *Recommended dietary nutrient intakes derived from Oftedal (1991) and NRC (2003).

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; AP, available protein; CP, crude protein; NDF, neutral detergent fiber; TNC, total nonstructural carbohydrates.

TABLE 2 Pairwise comparisons using a Tukey-Kramer adjustment for macronutrient contents of major food types consumed by *V. variegata* from January to December 2018.

Food type comparison		Variable Lipid	CP	AP	NDF	ADF	ADL	TNC
Difference	FR-FL	−3.374	−1.922	−1.340	1.382	−0.474	−1.943	4.901
	LE-FL	−5.387	3.297	2.441	5.015	1.658	0.301	−5.270
	PE-FL	−6.753	−3.242	−2.200	6.093	4.735	−11.189	−5.511
	LE-FR	−2.013	5.220	3.781	3.633	2.132	2.245	−10.172
	PE-FR	−3.379	−1.319	−0.856	4.711	5.209	−9.245	−10.412
	PE-LE	−1.366	−6.539	−4.637	1.078	3.077	−11.490	−0.241
p Value	FR-FL	0.285	0.848	0.953	0.997	0.999	0.955	0.770
	LE-FL	**0.033**	0.522	0.789	0.885	0.993	0.999	0.740
	PE-FL	0.181	0.861	0.989	0.955	0.971	0.331	0.925
	LE-FR	0.441	0.015	0.213	0.876	0.962	0.838	**0.034**
	PE-FR	0.673	0.985	0.999	0.972	0.950	0.417	0.573
	PE-LE	0.969	0.316	0.903	0.999	0.989	0.236	0.999

Note: Significant comparisons, after Bonferroni corrections, are in bold.

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; AP, available protein; CP, crude protein; FL, flower; FR, fruit; LE, leaf; NDF, neutral detergent fiber; PE, petiole; TNC, total nonstructural carbohydrates.

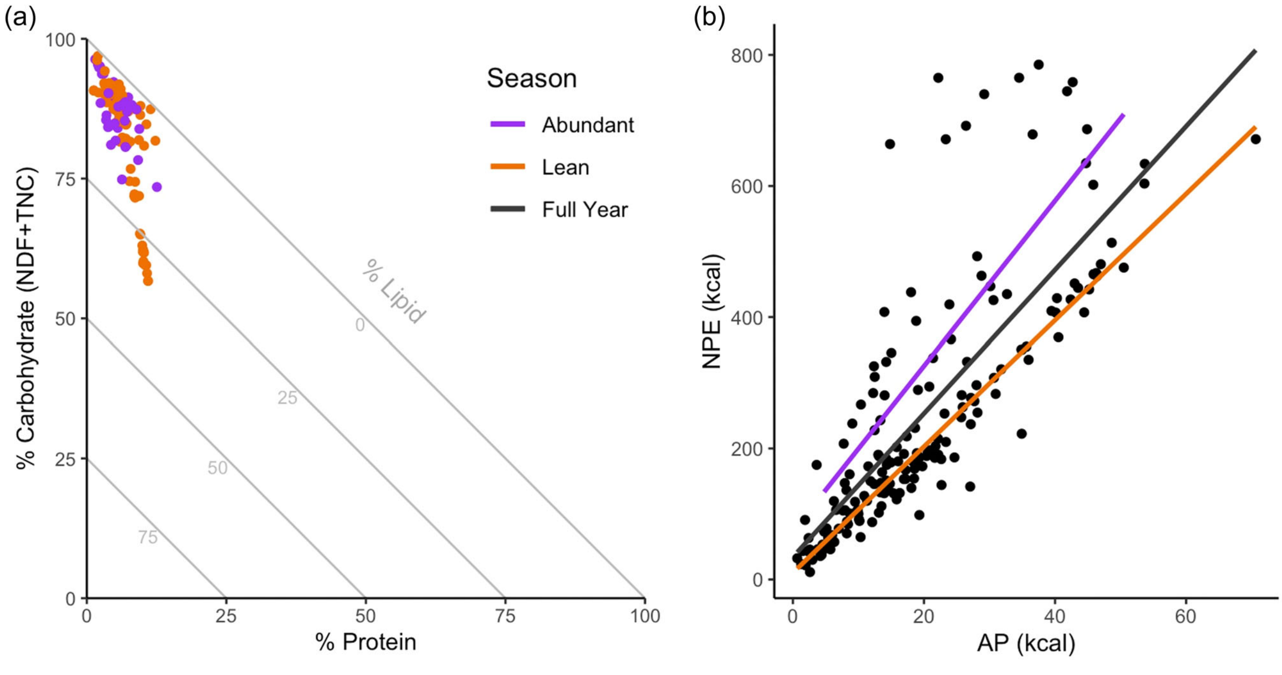

FIGURE 2 (a) Right-angled mixture triangle showing relative contributions of available protein (AP: *x* axis), carbohydrates (*y*-axis) and lipids (implicit axis) to daily metabolizable energy intake in the *Varecia variegata* diet. Each point represents the relative contributions of each macronutrient type to the total metabolizable energy intake in kcal on a given day. (b) Nonprotein energy (NPE) to available protein (AP) balancing in the *V. variegata* diet. Each point represents the total NPE and AP intake in kcal on a given day. Regression lines indicate the average ratios of NPE:AP in the diet.

day, with a mean of 440 kcal per day. *Varecia* gain most of their metabolizable energy (kcal) from carbohydrates (TNC + NDF; mean: 85%, range: 57%–97%) and relatively little from protein (AP; mean: 6%, range: 1%–13%). Lipids generally contributed little to the diet (mean: 9%, range: 1%–32%), however they substantially increased during parts of the lean season (Figure 2a). Overall, *Varecia* chose food items that were relatively consistent in macronutrient contents, occupying a small area of nutritional space.

Varecia balanced NPE:AP intakes in their annual daily diet at a ratio of 11:1 (based on regression coefficient of 11; Figure 2b). There is

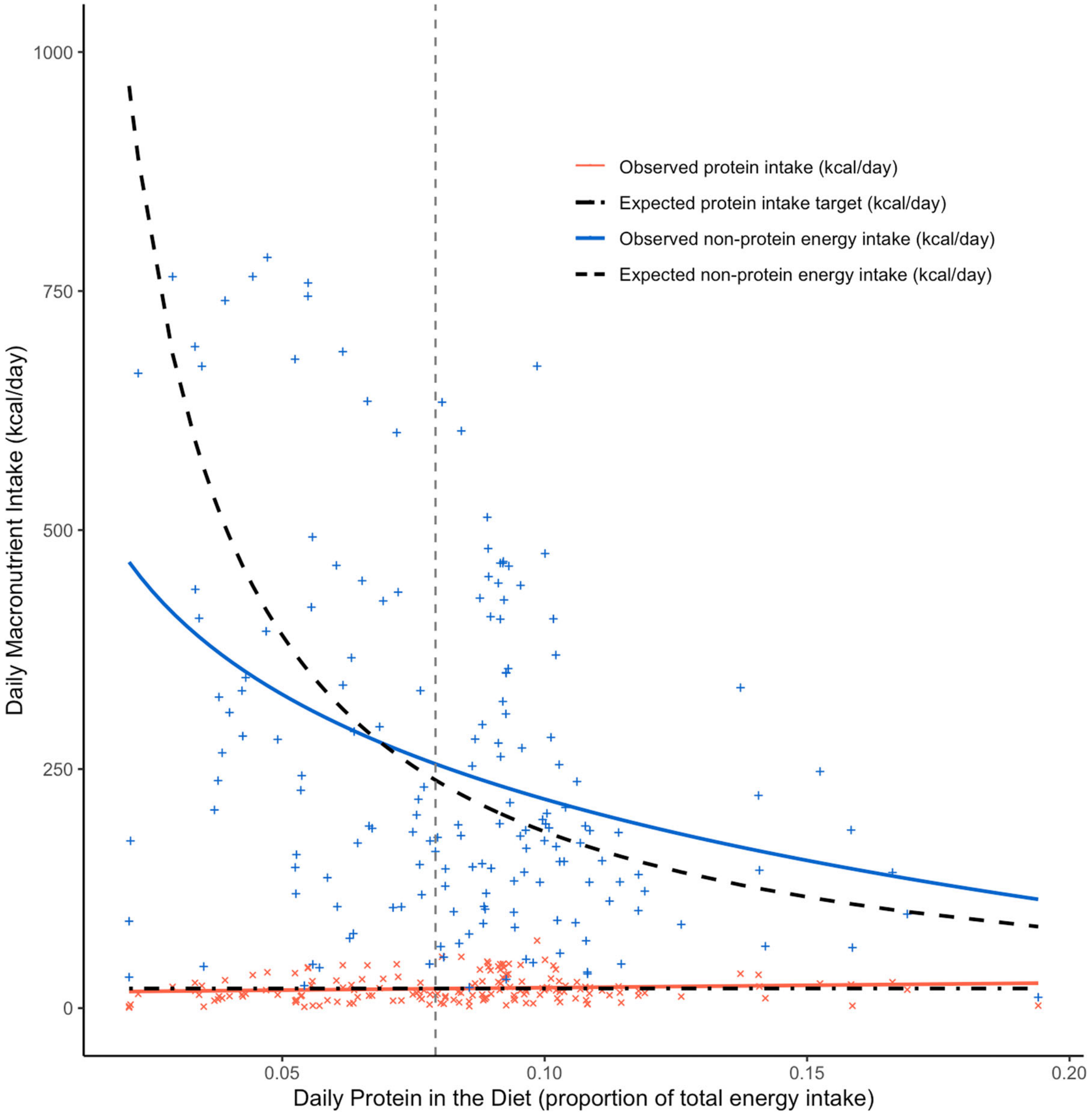

FIGURE 3 Observed versus expected protein and nonprotein energy intakes based on daily proportion of protein in the diet. Incomplete protein prioritization in the *Varecia variegata* diet is represented by nonprotein energy intake as a significant negative function of dietary proportion of protein. The vertical dashed gray line represents mean protin intake.

seasonal variation in balancing, with abundant season ratios falling higher (12.6:1) than lean season ratios (9.6:1). Furthermore, *Varecia* apparently regulated daily AP intake, and allowed daily NPE intake to vary considerably across the year (Figure 3). NPE intake was a negative function of dietary proportion of protein (linear regression: $R^2 = 0.129$, $F_{1,\ 166} = 24.59$, $p < 0.0001$; power function: $R^2 = 0.069$, $F_{1,\ 166} = 12.31$, $p < 0.0005$, with $L = -0.529$), indicating significant though incomplete protein prioritization (Figure 3) according to the range provided by Raubenheimer & Simpson, 2019).

Across the 168 sampling days, daily protein intake varied the least between 2 and 114 kcal per day, with a mean daily intake of 33.3 kcal (coefficient of variation [CV] = 0.669; Table 3). Daily lipid intake varied most (CV = 1.042), followed by neutral

TABLE 3 Variation in daily macronutrient and metabolizable energy intakes (all measured in kcal).

Nutrient	Mean	SD	CV
Available protein	33	13.7	0.669
Metabolizable energy	440	198.3	0.732
Nonprotein energy	421	189.7	0.733
Total nonstructural carbohydrate	61	32.0	0.850
Neutral detergent fiber	68	36.3	0.865
Lipid	13	8.6	1.042

Note: Nutrient components are ordered by increasing CV values.
Abbreviation: CV, coefficient of variation.

detergent fiber (CV = 0.865), and TNC (CV = 0.850), all major components of NPE. Daily NPE intakes varied between 18 and 1275 kcal per day (NPE CV = 0.733). This supports the patterns illustrated in Figure 3, that protein prioritization is partially occurring, but that the relationship is not strong.

Both dietary constituents and macronutrient intakes varied throughout the year. We found seasonal differences in the proportions of food types that contributed to the diet, based on dry matter mass of foods ingested in grams per day (fruits: ab = 78%, le = 35%; flowers: ab = 11%, le = 25%; leaves: ab = 8%, le = 36%). During the abundant season (November to April), *Varecia* also consumed greater daily caloric intakes of almost all nutrients (ME: ab = 568 kcal, le = 338 kcal; AP: ab = 36 kcal, le = 31 kcal; NPE: ab = 578 kcal, le = 319 kcal; TNC: ab = 96 kcal, le = 39 kcal; NDF: ab = 94 kcal, le = 52 kcal), with the exception of lipid intakes which were consistently low across seasons (ab = 13 kcal, le = 13 kcal). The percentage difference between calorie intakes across seasons is substantial, mainly due to differences in NPE intakes (40%), whereas protein intake is much less variable (14%).

Season had a significant effect on all nutrient components except NPE:AP ratio and lipid intakes, which were significantly predicted by both season ($p < 0.0001$) and frugivory ($p < 0.0001$), and AP intakes which were significantly predicted by frugivory ($p < 0.0001$; Table 4). Additionally, the mass of food ingested in grams was significantly predicted by season ($p < 0.0001$), with intakes being lower during lean seasons.

4 | DISCUSSION

In this study, we combined observations of feeding behavior with laboratory analyses of macronutrient content of foods to test four hypotheses about the nutritional strategy of a specialist frugivore, the black-and-white ruffed lemur (*V. variegata*). Specifically, we aimed to gain insight into the nutrient balancing of a fruit-specialist living in a seasonal environment, and by extension, provide insight into how frugivorous lemurs survive in Madagascar.

4.1 | Dietary macronutrients

We found that compared to recommendations for nonhuman primates, both food items and daily diets were low in protein and high in carbohydrates, as expected, but were variable in lipid content (Table 1, Figure 2a; National Research Council, 2003; Oftedal, 1991). This partially supports our first hypothesis (H1) that *Varecia* consume a diet low in protein and high in carbohydrates and lipids, with major food types differing significantly in macronutrient proportions. Fiber contents were higher than expected for this species, which has a limited capacity for fiber-fermentation (Campbell et al., 2000; Edwards, 1995; Edwards & Ulrey, 1999). Additionally, though it is well-established that fruits are generally lower in protein than other food types (Lambert & Rothman, 2015), the differences we found were not as pronounced as expected (see Donati et al., 2017;

TABLE 4 Summary of LMM outputs examining the effects of season and frugivory on ME, NPE:AP ratio, NPE intake, AP intake, TNC intake, lipid intake, and NDF intake (as proportions of the diet), and mass of food ingested (g) per day.

Nutrient	Fixed effect	Estimate	SE	*df*	*t*-value	*p* Value
ME	Season	−195.174	34.380	165	−5.677	**<0.0001**
	Frugivory	−0.795	0.436	165	−1.824	0.070
NPE:AP	Season	−0.349	1.253	161	−0.279	0.781
	Frugivory	0.099	0.016	160	6.221	**<0.0001**
AP	Season	−9.833	2.428	165	−4.049	**<0.0001**
	Frugivory	−0.152	0.031	165	−4.954	**<0.0001**
NPE	Season	−192.510	32.623	165	−5.901	**<0.0001**
	Frugivory	−0.734	0.414	165	−1.774	0.078
TNC	Season	−33.175	5.128	161	−6.470	**<0.0001**
	Frugivory	0.043	0.065	160	0.658	0.511
Lipid	Season	−3.608	1.562	165	−2.310	**0.0221**
	Frugivory	−0.086	0.020	165	−4.354	**<0.0001**
NDF	Season	−28.344	6.469	165	−4.381	**<0.0001**
	Frugivory	−0.046	0.082	165	−0.564	0.574
Food (g)	Season	−67.998	12.084	165	−5.627	**<0.0001**
	Frugivory	−0.128	0.153	165	−0.836	0.404

Note: General model formula: nutrient ~ season + frugivory + (1|focal) + (1|sex). Significant predictors for each response variable are highlighted in bold.

Abbreviations: AP, available protein; *df*, degrees of freedom; ME, metabolizable energy; NDF, neutral detergent fiber; NPE, nonprotein energy; SE, standard error; TNC, total nonstructural carbohydrates.

Ganzhorn et al., 2009) and not statistically significant (Tables 1 and 2).

During periods of fruit-scarcity, *Varecia* have been found to shift their diets towards increased folivory (e.g., Beeby & Baden, 2021; Britt, 2000; Holmes et al., 2016). However, during these times, animals have often been observed to consume substantial quantities of flowers (monthly mean: 7%, maximum: 52%; Beeby, *unpublished data*). Compared to other foods, we found that some flowers eaten by *Varecia* are very high in lipids (e.g., *Mammea* sp., *Symphonia* sp.; Supporting Information: Table S1), an energetically dense macronutrient (Conklin-Brittain et al., 2006). Flowers are an irregular but important resource for several other primates (e.g., *Piliocolobus badius*: Dominy & Lucas, 2001; *Cercopithecus mitis*: Bryer, 2020; *Cercopithecus ascanius*: Ross et al., 2022), though their nutritional contributions to primate diets remain underexamined. Ross et al. (2022) found *Symphonia* flowers to be an important high-fat resource in an otherwise low-fat diet in *Cercopithecus ascanius*—similar to what we found for *Varecia*. These results suggest that flowers may be an important resource for *Varecia* and lipids in these flowers may provide a vital source of NPE during periods of preferred food scarcity (as also seen in *Nycticebus javanicus*: Cabana et al., 2017) or when high-carbohydrate foods are less abundant. In fact, *Varecia* engage in some carbohydrate-lipid switching during lean season months (Figure 2a). Availability of certain foods, such as lipid-rich flowers, may play a crucial role in enabling ruffed lemurs to survive these periods of lower fruit availability. Future studies should record bi-weekly phenology to capture the availability of short-lived food flushes, such as flowers—and closely monitor climate change effects on fruiting and flowering phenology (Butt et al., 2015; Chapman et al., 2005). In addition, future studies should attempt to collect additional samples of each food item to further confirm these findings and should differentiate between food types more specifically, such as young versus mature leaves and flower buds versus flowers.

4.2 | Nutrient balancing

Further, we found strong support for our second hypothesis (H2) that *Varecia* ingest a diet of NPE:AP at a ratio higher than observed in other primates, with *Varecia* specifically balancing these nutrients at an 11:1 ratio (12.6:1 during the abundant season and 9.6:1 during the lean season). This is the highest NPE:AP ratio observed in any primate for which data are available. The three previously highest ratios came from two predominantly frugivorous species (9.6:1 in *Ateles chamek*: Felton, Felton, Lindenmayer, et al., 2009; 5.7:1 in *Pan troglodytes*: Uwimbabazi et al., 2021) and one primate that is typically characterized as a folivore but often consumes large amounts of fruit (9.5:1 in *Propithecus diadema*: Irwin et al., 2015). Interestingly, one of the other primates with the highest NPE:AP ratio is also a lemur. Seemingly, these two species converge on similar optimum nutrient balances, despite having strikingly different dietary adaptations (Campbell et al., 2004). Lemurs may have limited ability to rely on protein as a source of calories, regardless of dietary guild or preferences, because protein is limited in Madagascar's environment (Donati et al., 2017; Ganzhorn et al., 2009).

In support of our third hypothesis (H3) that protein (AP) in the *Varecia* diet is tightly regulated, we found that daily protein intake varies least (Table 3). NPE intake was a negative function of the proportion of protein in the diet, indicating significant but not complete protein prioritization (Figure 3). Protein prioritization appears to be a common rule of compromise found in primates to date, particularly frugivores (Felton, Felton, Raubenheimer, et al., 2009; Takahashi et al., 2021), with several taxa maximizing NPE intake within this constraint (e.g., Uwimbabazi et al., 2021). Particularly interesting is that carbohydrates (CV: 0.850) and lipids (CV: 1.042), both individual components of NPE, show greater variation than overall NPE intakes, suggesting *Varecia* are interchanging NPE components in their diet to maximize intakes.

The intake targets for *Varecia* appear to be 9 kcal/kg AP and 115 kcal/kg NPE (based on observed long-term mean intakes as target divided by mean body mass of 3.65 kg: Baden et al., 2008). However, given that individuals did not reproduce during our study, these may be suboptimal and true intake requirements for reproduction may be higher. Mean protein intake was 33.3 kcal per day, equating to a mean of 6% protein contribution to daily metabolizable energy (Figure 2b), with contributions ranging from 1% to 13% of daily metabolizable energy intake. This is lower than the metabolizable energy gains from proteins seen in several other frugivorous primates (19% in *Cercopithecus mitis*: Takahashi et al., 2021; 15% in *Pan troglodytes*: Uwimbabazi et al., 2021), but close to 9% protein contribution documented in *Ateles chamek* (Felton, Felton, Raubenheimer, et al., 2009). Nevertheless, captive studies advise that nonreproductive adult primates require 5%–8% of their energy as protein (Oftedal, 1991). This would suggest ruffed lemurs acquire sufficient dietary protein, despite their specialization to consuming Madagascar's protein-limited fruits (Donati et al., 2017; Ganzhorn et al., 2009; Thurau et al., 2021).

4.3 | Seasonal variation

We found seasonal variation in NPE:AP balancing, with the ratio reaching 12.6:1 during the fruit-abundant season and dropping to 9.6:1 during the fruit-lean season (Figure 2a). The ratio of NPE:AP is significantly predicted by the degree of frugivory in the diet ($p < 0.0001$; Table 4), and the majority of seasonal variation in calorie intakes is due to differences in NPE intakes (40% difference). Moreover, metabolizable energy intake was not significantly predicted by season and instead by the degree of frugivory (Table 4). This differs to patterns seen in other lemurs, which maintain more constant proportions of NPE:AP across seasons at the expense of calories consumed during lean seasons (*Propithecus*: Irwin et al., 2014, 2015; *Lepilemur*: Dröscher et al., 2016). However, in *Propithecus*, calorie intake is positively correlated with the degree of frugivory, suggesting fruit consumption is a critical determinant of lemur nutritional outcomes (Irwin et al., 2014, 2015). As a fruit-specialist, *Varecia* may be less able to maintain strict balancing when preferred food availability fluctuates. Instead, when faced with variation in preferred food availability, they appear to maximize energy intakes where possible from NPE-rich fruit resources, while maintaining sufficient protein intakes. Other frugivores, such as *Pongo pygmaeus* (Harrison et al., 2010; Vogel et al., 2015) and *Ateles chamek* (Felton, Felton, Lindenmayer, et al., 2009; Felton, Felton, Raubenheimer, et al., 2009), exhibit similar seasonal effects, reducing calorie intake during fruit-lean seasons while also consuming diets with lower NPE:AP ratios.

4.4 | Relationship to food types

Finally, we hypothesized that (H4) daily NPE:AP ratio, and NPE, AP, TNC, and lipid intakes would vary as a function of the proportions of fruit, flowers, and leaves in the *Varecia* diet. The proportion of fruit in the daily diet best predicted NPE:AP ratio (Table 4), indicating changes in NPE:AP ratio are primarily driven by changes in fruit intake, as has been found in other primates (*Cercopithecus mitis*: Takahashi et al., 2021; *Pan troglodytes*: Uwimbabazi et al., 2021), including another lemur (*Propithecus diadema*: Irwin et al., 2014). During fruit-lean seasons, when *Varecia* supplement their diet with leaf material, their NPE:AP ratio fluctuates around a ratio of 9.7:1. However, during fruit-abundant seasons, when preferred foods (that they are best suited to digesting) are available, *Varecia* seem to maximize their calorie intakes by eating many food items high in NPE. Conversely, the proportion of leaves was the best fit model for NPE intake and TNC intake. The strongest predictor of daily AP intake and lipid intake was the proportion of flowers in the daily diet (Table 5). Lipid intake strongly positively correlated to flower consumption, which suggests that flowers, which are often consumed during the fruit-lean season, may play an important role in providing NPE when fruits are less available. These results suggest that no single food type has the strongest influence on variance in most macronutrient intakes. While we did not expect this to be the case, these results do make sense considering this species' fruit-specialization. Overall NPE:AP balance is best predicted by their preferred diet, which comprises mostly fruit; however deviations away from their preferred diet toward eating other food types can have a large impact on their intakes of specific nutrients, especially NPE.

4.5 | Synthesis and future directions

In this study we demonstrated that, despite its fruit-specialization, the black-and-white ruffed lemur (*V. variegata*) balances nutrients within Madagascar's protein-limited environment, and uses an incomplete protein prioritization strategy. Our results contribute an important addition to the growing body of literature examining nutrient balancing among primates, and how different taxa respond to environmental drivers of variation in nutritional balance. Moreover, these data provide insights into the different constraints animals in different geographic regions are subject to. Notably, Madagascar's lemurs are subject to significant protein limitation (Donati et al., 2017; Ganzhorn et al., 2009; Thurau et al., 2021), leading to sometimes divergent nutrient strategies than expected based on other primates in similar guilds (as seen in *Propithecus diadema*: Irwin et al., 2015). Evidently, Madagascar's lemurs are extremely constrained in their ability to acquire sufficient protein and therefore must prioritize protein to some degree regardless of dietary preference or adaptations. These effects may be particularly pronounced in fruit-specialist taxa that are poorly suited to the fiber fermentation required to digest leaves (Campbell et al., 2004; Edwards & Ulrey, 1999) and gain most of their energy intake from high NPE, low AP fruits. As such, maintaining sufficient nutrient intakes may be precarious and these specialist consumers may be at greater risk of consuming inadequate nutrient balances due to irregularities in fruiting phenology and other environmental stochasticities resulting from climate change (Chapman et al., 2005; Dunham et al., 2018; Renner & Zohner, 2018; Rothman et al., 2015; Wright, 2006). Furthermore, certain unusual behavioral and life history traits of *Varecia*, such as fission-fusion social dynamics (Baden et al., 2016), "boom-bust" reproduction (Baden et al., 2013; Ratsimbazafy, 2002) and communal infant rearing (Baden et al., 2013; Baden, 2011, 2019) may be driven by their ability to meet their nutritional needs during certain times of year. Weaning coincides with peak fruit availability, meaning that females are gestating during an energetically challenging time (Baden, 2011; Baden et al., 2016; Wright, 1999). With climate change, there is a growing concern of an evolutionary mismatch, where phenology and life history are no longer in sync (Wright, 1999, 2006), which may constrain their ability to switch to other forms of NPE during energetically challenging periods (e.g., peak lactation, weaning) and have lasting repercussions for the species. Relating nutritional data to these parameters will enable us to further untangle the relationships between ecology, behavior, and physiology in this unique taxon and other fruit-specialists living in highly seasonal, nutrient-scarce, and changing landscapes.

AUTHOR CONTRIBUTIONS

Nina Beeby: Conceptualization (equal); data curation (lead); formal analysis (lead); funding acquisition (supporting); investigation (lead); methodology (supporting); project administration (lead); visualization (lead); writing—original draft (lead); writing—review and editing (lead). **Jessica M. Rothman**: Conceptualization (supporting); formal analysis (supporting); investigation (supporting); methodology (lead); project administration (supporting); resources (equal); supervision (equal); visualization (supporting); writing—review and editing (equal). **Andrea L. Baden**: Conceptualization (equal); formal analysis (supporting); funding acquisition (lead); investigation (supporting); project administration (supporting); resources (equal); supervision (equal); writing—review and editing (equal).

ACKNOWLEDGMENTS

Thank you to ICTE, MICET, and Centre ValBio for facilitating our research in Madagascar, and Madagascar National Parks (MNP) for permission to conduct our research in Ranomafana National Park. We thank Lahitsara Pierre, Razafindraibe Jean Guy, Rakotonjatovo Justin, Tombotiana Aime Victor, Leroa, and Velomaro for their expertise in the field. We also thank Giulia Rossi and Lotte van den Hout for assistance during data collection and Jenny Paltan for invaluable training and assistance during data analyses. We thank Erin Vogel for helpful discussions. We are grateful for the comments of the anonymous reviewers who helped us to improve the manuscript. Funding was generously provided by The Leakey Foundation (ALB), PSC-CUNY (ALB), Hunter College—CUNY (ALB, JMR), and The Graduate Center—CUNY (NB). Research complied with the laws and guidelines set forth by ANGAP/Madagascar National Parks and Hunter College IACUC (#AB-lemurs 5/20-01) and adhered to the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Nonhuman Primates.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available upon reasonable request by the corresponding author.

ORCID

Nina Beeby http://orcid.org/0000-0001-9918-8135

REFERENCES

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, *49*, 227–266.

Baden, A. L., Brenneman, R. A., & Louis, Jr., E. E. (2008). Morphometrics of wild black-and-white ruffed lemurs [*Varecia variegata*; Kerr, 1792]. *American Journal of Primatology*, *70*(10), 913–926.

Baden, A. L. (2011). *Communal infant care in black-and-white ruffed lemurs (Varecia variegata)* [PhD dissertation]. Stony Brook University.

Baden, A. L. (2019). A description of nesting behaviors, including factors impacting nest site selection, in black-and-white ruffed lemurs (*Varecia variegata*). *Ecology and Evolution*, *9*(3), 1010–1028.

Baden, A. L., Wright, P. C., Louis, E. E., & Bradley, B. J. (2013). Communal nesting, kinship, and maternal success in a social primate. *Behavioral Ecology and Sociobiology*, *67*, 1939–1950.

Baden, A. L., Webster, T. H., & Kamilar, J. M. (2016). Resource seasonality and reproduction predict fission–fusion dynamics in black-and-white ruffed lemurs (*Varecia variegata*). *American Journal of Primatology*, *78*, 256–279.

Baden, A. L., Oliveras, J., & Gerber, B. D. (2020). Sex-segregated range use by black-and-white ruffed lemurs (*Varecia variegata*) in Ranomafana National Park, Madagascar. *Folia Primatologica*, *92*(1), 12–34.

Balko, E. A. (1998). *A behaviorally plastic response to forest composition and logging disturbance by Varecia variegata variegata in Ranomafana National Park, Madagascar* [Dissertation]. State University of New York.

Balko, E. A., & Brian Underwood, H. (2005). Effects of forest structure and composition on food availability for *Varecia variegata* at Ranomafana National Park, Madagascar. *American Journal of Primatology*, *66*, 45–70.

Barboza, P. S., Parker, K. L. & Hume, I. D., (Eds.). (2009). *Integrative wildlife nutrition*. Springer Berlin Heidelberg.

Beeby, N., & Baden, A. L. (2021). Seasonal variability in the diet and feeding ecology of black-and-white ruffed lemurs (*Varecia variegata*) in Ranomafana National Park, southeastern Madagascar. *American Journal of Physical Anthropology*, *174*, 763–775.

Belovsky, G. E. (1984). Herbivore optimal foraging: A comparative test of three models. *The American Naturalist*, *124*(1), 97–115.

Britt, A. (2000). Diet and feeding behaviour of the black-and-white ruffed lemur (*Varecia variegata variegata*) in the Betampona Reserve, Eastern Madagascar. *Folia Primatologica*, *71*, 133–141.

Brockman, D. K., & Van Schaik, C. P. (Eds.). (2005). *Seasonality in primates: Studies of living and extinct human and non-human primates* (Vol. 44). Cambridge University Press.

Brown, J. L., & Yoder, A. D. (2015). Shifting ranges and conservation challenges for lemurs in the face of climate change. *Ecology and Evolution*, *5*(6), 1131–1142.

Bryer, M. (2020). *Nutritional strategy and social environment in redtail monkeys* (Cercopithecus ascanius). CUNY.

Butt, N., Seabrook, L., Maron, M., Law, B. S., Dawson, T. P., Syktus, J., & McAlpine, C. A. (2015). Cascading effects of climate extremes on vertebrate fauna through changes to low-latitude tree flowering and fruiting phenology. *Global Change Biology*, *21*(9), 3267–3277.

Cabana, F., Dierenfeld, E., Wirdateti, W., Donati, G., & Nekaris, K. A. I. (2017). The seasonal feeding ecology of the javan slow loris (*Nycticebus javanicus*): Cabana et al. *American Journal of Physical Anthropology*, *162*, 768–781.

Campbell, J. L., Eisemann, J. H., Williams, C. V., & Glenn, K. M. (2000). Description of the gastrointestinal tract of five lemur species: *Propithecus tattersalli*, *Propithecus verreauxi coquereli*, *Varecia variegata*, *Hapalemur griseus*, and *Lemur catta*. *American Journal of Primatology*, *52*, 133–142.

Campbell, J. L., Williams, C. V., & Eisemann, J. H. (2004). Use of total dietary fiber across four lemur species (Propithecus verreauxi coquereli, Hapalemur griseus griseus, Varecia variegata, and Eulemur fulvus): does fiber type affect digestive efficiency? *American Journal of Primatology*, *64*, 323–335.

Chapman, C. A., Chapman, L. J., Struhsaker, T. T., Zanne, A. E., Clark, C. J., & Poulsen, J. R. (2005). A long-term evaluation of fruiting phenology: Importance of climate change. *Journal of Tropical Ecology*, *21*(1), 31–45.

Conklin-Brittain, N. L., Knott, C. D., & Wrangham, R. W. (2006). Energy intake by wild chimpanzees and orangutans: Methodological considerations and a preliminary comparison. In G. Hohmann, M. M. Robbins & C. Boesch, (Eds.), *Feeding ecology in apes and other primates. Ecological, physical and behavioral aspects* (pp. 445–571). Cambridge University Press.

Cui, Z. W., Wang, Z. L., Shao, Q., Raubenheimer, D., & Lu, J. Q. (2018). Macronutrient signature of dietary generalism in an ecologically diverse primate in the wild. *Behavioral Ecology*, *29*, 804–813.

Cui, Z., Shao, Q., Grueter, C. C., Wang, Z., Lu, J., & Raubenheimer, D. (2019). Dietary diversity of an ecological and macronutritional generalist primate in a harsh high-latitude habitat, the Taihangshan macaque (*Macaca mulatta tcheliensis*). *American Journal of Primatology*, *81*, e22965.

DeGabriel, J. L., Moore, B. D., Felton, A. M., Ganzhorn, J. U., Stolter, C., Wallis, I. R., Johnson, C. N., & Foley, W. J. (2014). Translating nutritional ecology from the laboratory to the field: Milestones in linking plant chemistry to population regulation in mammalian browsers. *Oikos*, *123*(3), 298–308.

Delorme, M., & Thomas, D. W. (1999). Comparative analysis of the digestive efficiency and nitrogen and energy requirements of the phyllostomid fruit-bat (*Artibeus jamaicensis*) and the pteropodid fruit-bat (*Rousettus aegyptiacus*). *Journal of Comparative Physiology B:*

Biochemical, Systemic, and Environmental Physiology, *169*(2), 123–132.

Dew, J. L., & Wright, P. (1998). Frugivory and seed dispersal by four species of primates in Madagascar's Eastern Rain Forest. *Biotropica*, *30*, 425–437.

Dewar, R. E., & Richard, A. F. (2007). Evolution in the hypervariable environment of Madagascar. *Proceedings of the National Academy of Sciences*, *104*, 13723–13727.

Dominy, N. J., & Lucas, P. W. (2001). Ecological importance of trichromatic vision to primates. *Nature*, *410*(6826), 363–366.

Donati, G., Santini, L., Eppley, T. M., Arrigo-Nelson, S. J., Balestri, M., Boinski, S., Bollen, A., Bridgeman, L. L., Campera, M., Carrai, V., Chalise, M. K., Derby Lewis, A., Hohmann, G., Kinnaird, M. F., Koenig, A., Kowalewski, M., Lahann, P., McLennan, M. R., Nekaris, A. K. I., … Ganzhorn, J. U. (2017). Low levels of fruit nitrogen as drivers for the evolution of Madagascar's primate communities. *Scientific Reports*, *7*, 14406.

Dröscher, I., Rothman, J. M., Ganzhorn, J. U., & Kappeler, P. M. (2016). Nutritional consequences of folivory in a small-bodied lemur (*Lepilemur leucopus*): Effects of season and reproduction on nutrient balancing: Folivory in a small-bodied lemur. *American Journal of Physical Anthropology*, *160*, 197–207.

Dunham, A. E., Razafindratsima, O. H., Rakotonirina, P., & Wright, P. C. (2018). Fruiting phenology is linked to rainfall variability in a tropical rain forest. *Biotropica*, *50*, 396–404.

Dunham, N. T., & Rodriguez-Saona, L. E. (2018). Nutrient intake and balancing among femalecolobus angolensis palliatusinhabiting structurally distinct forest areas: Effects of group, season, and reproductive state. *American Journal of Primatology*, *80*, e22878.

Edwards, M. S. (1995). *Comparative adaptations to folivory in primates* [PhD dissertation]. Michigan State University.

Edwards, M. S., & Ullrey, D. E. (1999). Effect of dietary fiber concentration on apparent digestibility and digesta passage in non-human primates. I. Ruffed lemurs (*Varecia variegata variegata* and *V. v. rubra*). *Zoo Biology*, *18*, 529–536.

Federman, S., Sinnott-Armstrong, M., Baden, A. L., Chapman, C. A., Daly, D. C., Richard, A. R., Valenta, K., & Donoghue, M. J. (2017). The paucity of frugivores in Madagascar may not be due to unpredictable temperatures or fruit resources. *PLoS One*, *12*, e0168943.

Felton, A. M., Felton, A., Lindenmayer, D. B., & Foley, W. J. (2009). Nutritional goals of wild primates. *Functional Ecology*, *23*, 70–78.

Felton, A. M., Felton, A., Raubenheimer, D., Simpson, S. J., Foley, W. J., Wood, J. T., & Lindenmayer, D. B. (2009). Protein content of diets dictates the daily energy intake of a free-ranging primate. *Behavioral Ecology*, *20*, 685–690.

Ganzhorn, J. U., Arrigo-Nelson, S., Boinski, S., Bollen, A., Carrai, V., Derby, A., Donati, G., Koenig, A., Kowalewski, M., Lahann, P., Norscia, I., Polowinsky, S. Y., Schwitzer, C., Stevenson, P. R., Talebi, M. G., Tan, C., Vogel, E. R., & Wright, P. C. (2009). Possible fruit protein effects on primate communities in Madagascar and the Neotropics. *PLoS One*, *4*, e8253.

Galetti, M., Keuroghlian, A., Hanada, L., & Morato, M. I. (2001). Frugivory and seed dispersal by the Lowland Tapir (*Tapirus terrestris*) in Southeast Brazil. *Biotropica*, *33*(4), 723–726.

Gould, L., Sussman, R. W., & Sauther, M. L. (1999). Natural disasters and primate populations: The effects of a 2-year drought on a naturally occurring population of ring-tailed lemurs (*Lemur catta*) in southwestern Madagascar. *International Journal of Primatology*, *20*, 69–84.

Harrison, M. E., Morrogh-Bernard, H. C., & Chivers, D. J. (2010). Orangutan energetics and the influence of fruit availability in the nonmasting peat-swamp forest of Sabangau, Indonesian Borneo. *International Journal of Primatology*, *31*(4), 585–607.

Hemingway, C. A. (1996). Morphology and phenology of seeds and whole fruit eaten by Milne-Edwards' Sifaka, *Propithecus diadema edwardsi* in Ranomafana National Park, Madagascar. *International Journal of Primatology*, *17*, 637–659.

Hemingway, C. A., & Bynum, N. (2005). The influence of seasonality on primate diet and ranging. In D. K. Brockman & C. P. van Schaik, (Eds.), *Seasonality in primates: Studies of living and extinct human and nonhuman primates* (pp. 57–104). Cambridge University Press.

Herrera, G. L., Gutierrez, E., Hobson, K. A., Altube, B., Díaz, W. G., & Sánchez-Cordero, V. (2002). Sources of assimilated protein in five species of New World frugivorous bats. *Oecologia*, *133*(3), 280–287.

Herrera M, L. G., Rodríguez G, M., & Hernández P, P. (2009). Sources of assimilated protein in a specialized tropical frugivorous bird, the yellow-throated Euphonia (*Euphonia hirundinacea*). *The Auk*, *126*(1), 175–180.

Holmes, S. M., Gordon, A. D., Louis, E. E., Jr., & Johnson, S. E. (2016). Fission-fusion dynamics in black-and-white ruffed lemurs may facilitate both feeding strategies and communal care of infants in a spatially and temporally variable environment. *Behavioral Ecology and Sociobiology*, *70*, 1949–1960.

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 diademed sifakas. *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.

Izhaki, I., & Safriel, U. N. (1989). Why are there so few exclusively frugivorous birds? Experiments on fruit digestibility. *Oikos*, *54*, 23–32.

Janson, C. H., & Chapman, C. A. (1999). Resources and primate community structure. In J. G. Fleagle, C. Janson & K. Reed, (Eds.), *Primate communities* (pp. 237–267). Cambridge University Press.

Johnson, C. A., Raubenheimer, D., Chapman, C. A., Tombak, K. J., Reid, A. J., & Rothman, J. M. (2017). Macronutrient balancing affects patch departure by guerezas (Colobus guereza). *American Journal of Primatology*, *79*, 1–9.

Johnson, C. A., Raubenheimer, D., Rothman, J. M., Clarke, D., & Swedell, L. (2013). 30 days in the life: Daily nutrient balancing in a wild chacma baboon. *PLoS One*, *8*, e70383.

Karr, J. R., Robinson, S. K., Blake, J. G., & Bierregaard, Jr., R. O. (1990). Birds of four Neotropical forests. In A. Gentry (Ed.), *Four Neotropical Forests* (pp. 237–259). Yale University Press.

Lambert, J. E. (1998). Primate digestion: Interactions among anatomy, physiology, and feeding ecology. *Evolutionary Anthropology: Issues, News, and Reviews*, *7*, 8–20.

Lambert, J. E., Fellner, V., McKenney, E., & Hartstone-Rose, A. (2014). Binturong (*Arctictis binturong*) and kinkajou (*Potos flavus*) digestive strategy: Implications for interpreting frugivory in Carnivora and Primates. *PLoS One*, *9*(8), e105415.

Lambert, J. E., & Rothman, J. M. (2015). Fallback foods, optimal diets, and nutritional targets: Primate responses to varying food availability and quality. *Annual Review of Anthropology*, *44*, 493–512.

Lewis, R. J., & Bannar-Martin, K. H. (2012). The impact of cyclone Fanele on a tropical dry forest in Madagascar. *Biotropica*, *44*, 135–140.

MacArthur, R. H., & Pianka, E. R. (1966). On optimal use of a patchy environment. *The American Naturalist*, *100*(916), 603–609.

Martinez, B. T., & Razafindratsima, O. H. (2014). Frugivory and seed dispersal patterns of the red-ruffed lemur, *Varecia rubra*, at a forest restoration site in Masoala National Park, Madagascar. *Folia Primatologica*, *85*, 228–243.

Martínez-Mota, R., Righini, N., & Palme, R. (2016). Fluctuations in daily energy intake do not cause physiological stress in a Neotropical primate living in a seasonal forest. *Oecologia*, *182*(4), 973–984.

McNab, B. K. (2002). *The physiological ecology of vertebrates: A view from energetics* (p. 523). Cornell University Press.

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *Journal of AOAC International*, *85*, 1217–1240.

Morelli, T. L., Smith, A. B., Mancini, A. N., Balko, E. A., Borgerson, C., Dolch, R., Farris, Z., Federman, S., Golden, C. D., Holmes, S. M., Irwin, M., Jacobs, R. L., Johnson, S., King, T., Lehman, S. M., Louis, E. E., Murphy, A., Randriahaingo, H. N. T., Randrianarimanana, H. L. L., … Baden, A. L. (2020). The fate of Madagascar's rainforest habitat. *Nature Climate Change*, *10*, 89–96.

National Research Council. (2003). *Nutrient requirements of nonhuman primates* (Second Revised Edition.). The National Academies Press.

Oftedal, O. T. (1991). The nutritional consequences of foraging in primates: The relationship of nutrient intakes to nutrient requirements. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *334*(1270), 161–170.

Oftedal, O. T., Baer, D. J., & Allen, M. E. (1996). The feeding and nutrition of herbivores. In D. G. Kleiman, K. V. Thompson, & C. K. Baer (Eds.) *Wild mammals in captivity: Principles and techniques for Zoo Management* (pp. 129–138). University of Chicago Press.

Pyke, G. H. (1984). Optimal foraging theory: A critical review. *Annual Review of Ecology and Systematics*, *15*, 523–575.

Ratsimbazafy, J. H. (2002). *On the brink of extinction and the process of recovery: Responses of black-and-white ruffed lemurs (Varecia variegata variegata) to disturbance in Manombo* [Dissertation]. Stony Brook University.

Ratsimbazafy, J. H. (2006). Diet composition, foraging, and feeding behavior in relation to habitat disturbance: Implications for the adaptability of ruffed lemurs (*Varecia variegata editorium*) in Manombo Forest, Madagascar. In L. Gould, & M. L. Sauther (Eds.), *Lemurs. Developments in primatology: Progress and prospect* (pp. 403–422). Springer.

Raubenheimer, D. (2011). Toward a quantitative nutritional ecology: The right-angled mixture triangle. *Ecological Monographs*, *81*, 407–427.

Raubenheimer, D., Simpson, S. J., & Mayntz, D. (2009). Nutrition, ecology and nutritional ecology: Toward an integrated framework. *Functional Ecology*, *23*, 4–16.

Raubenheimer, D., & Simpson, S. J. (2004). Organismal stoichiometry: Quantifying non-independence among food components. *Ecology*, *85*(5), 1203–1216.

Raubenheimer, D., & Simpson, S. J. (2016). Nutritional ecology and human health. *Annual Review of Nutrition*, *36*, 603–626.

Raubenheimer, D., & Simpson, S. J. (2019). Protein leverage: Theoretical foundations and ten points of clarification. *Obesity*, *27*(8), 1225–1238.

Razafindratsima, O. H., & Dunham, A. E. (2016). Retracted: Co-fruiting plant species share similar fruit and seed traits while phylogenetic patterns vary through time. *Journal of Ecology*, *104*, 1789–1798.

Razafindratsima, O. H., Jones, T. A., & Dunham, A. E. (2014). Patterns of movement and seed dispersal by three lemur species. *American Journal of Primatology*, *76*, 84–96.

Renner, S. S., & Zohner, C. M. (2018). Climate change and phenological mismatch in trophic interactions among plants, insects, and vertebrates. *Annual Review of Ecology, Evolution, and Systematics*, *49*(1), 165–182.

Robbins, C. (2012). *Wildlife feeding and nutrition*. Elsevier.

Rode, K. D., & Robbins, C. T. (2000). Why bears consume mixed diets during fruit abundance. *Canadian Journal of Zoology*, *78*(9), 1640–1645.

Ross, A. C., Bryer, M. A. H., Chapman, C. A., Rothman, J. M., Nevo, O., & Valenta, K. (2022). Why eat flowers? Symphonia globulifera flowers provide a fatty resource for red-tailed monkeys. *Folia Primatologica*, *93*(1), 41–52.

Rothman, J. M., Raubenheimer, D., & Chapman, C. A. (2011). Nutritional geometry: Gorillas prioritize non-protein energy while consuming surplus protein. *Biology Letters*, *7*, 847–849.

Rothman, J. M., Chapman, C. A., & van Soest, P. J. (2012). Methods in primate nutritional ecology. *International Journal of Primatology*, *33*, 542–566.

Rothman, J. M., Chapman, C. A., Struhsaker, T. T., Raubenheimer, D., Twinomugisha, D., & Waterman, P. G. (2015). Long-term declines in nutritional quality of tropical leaves. *Ecology*, *96*(3), 873–878.

Saldaña-Vázquez, R. A., Ruiz-Sanchez, E., Herrera-Alsina, L., & Schondube, J. E. (2015). Digestive capacity predicts diet diversity in Neotropical frugivorous bats. *Journal of Animal Ecology*, *84*(5), 1396–1404.

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.

Simpson, S. J., Batley, R., & Raubenheimer*, D. (2003). Geometric analysis of macronutrient intake in humans: The power of protein? *Appetite*, *41*, 123–140.

Stephens, D. W., & Krebs, J. R. (2019). *Foraging theory*. Princeton University Press.

Takahashi, M. Q., Rothman, J. M., Raubenheimer, D., & Cords, M. (2021). Daily protein prioritization and long-term nutrient balancing in a dietary generalist, the blue monkey. *Behavioral Ecology*, *32*, 223–235.

Terborgh, J. (1986). Community aspects of frugivory in tropical forests, A. Estrada, & T. H. Fleming (Eds.), *Frugivores and seed dispersal* (pp. 371–384). Springer.

Thomas, D. W. (1984). Fruit intake and energy budgets of frugivorous bats. *Physiological Zoology*, *57*(4), 457–467.

Thurau, E. G., Rahajanirina, A. N., & Irwin, M. T. (2021). Condensed tannins in the diet of folivorous diademed sifakas and the gap between crude and available protein. *American Journal of Primatology*, *83*(3), e23239.

Uwimbabazi, M., Raubenheimer, D., Tweheyo, M., Basuta, G. I., Conklin-Brittain, N. L., Wrangham, R. W., & Rothman, J. M. (2021). Nutritional geometry of female chimpanzees (*Pan troglodytes*). *American Journal of Primatology*, *83*, e23269.

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597.

Vasey, N. (2006). Impact of seasonality and reproduction on social structure, ranging patterns and fission–fusion social organization in red ruffed lemurs. In L. Gould, & M. L. Sauther (Eds.) *Lemurs: Ecology and adaptation* (pp. 275–305). Springer/Kluwer.

Vieilledent, G., Grinand, C., Rakotomalala, F. A., Ranaivosoa, R., Rakotoarijaona, J. R., Allnutt, T. F., & Achard, F. (2018). Combining global tree cover loss data with historical national forest cover maps to look at six decades of deforestation and forest fragmentation in Madagascar. *Biological Conservation*, *222*, 189–197.

Vogel, E. R., Harrison, M. E., Zulfa, A., Bransford, T. D., Alavi, S. E., Husson, S., Morrogh-Bernard, H., Santiano, Firtsman, T., Utami-Atmoko, S. S., van Noordwijk, M. A., & Farida, W. R. (2015). Nutritional differences between two orangutan habitats: Implications for population density. *PLoS One*, *10*(10), e0138612.

White, F. J., Overdorff, D. J., Balko, E. A., & Wright, P. C. (1995). Distribution of ruffed lemurs (Varecia variegata) in Ranomafana National Park, Madagascar. *Folia Primatologica*, *64*, 124–131.

Wright, P. C. (1999). Lemur traits and Madagascar ecology: Coping with an Island environment. *American Journal of Physical Anthropology*, *110*, 31–72.

Wright, P. C. (2006). Considering climate change effects in lemur ecology and conservation. In L. Gould, & M. L. Sauther (Eds.) *Lemurs: Ecology and adaptation* (pp. 385–401). Springer.

Wright, P. C., Razafindratsita, V. R., Pochron, S. T., & Jernvall, J. (2005). The key to Madagascar frugivores. In J. L. Dew, & J. P. Boubli (Eds.), *Tropical fruits and frugivores* (pp. 121–138). Springer.

Wright, P. C., Tecot, S. R., Erhart, E. M., Baden, A. L., King, S. J., & Grassi, C. (2011). Frugivory in four sympatric lemurs: Implications for the future of Madagascar's forests. *American Journal of Primatology*, *73*, 585–602.

Wright, P. C., Erhart, E. M., Tecot, S., Baden, A. L., Arrigo-Nelson, S. J., Herrera, J., & Zohdy, S. (2012). Long-term lemur research at

Centre ValBio, Ranomafana National Park, Madagascar. In P. Kappeler, & D. Watts (Eds.), *Long-term field studies of primates* (pp. 67–100). Springer.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Beeby, N., Rothman, J. M., & Baden, A. L. (2023). Nutrient balancing in a fruit-specialist primate, the black-and-white ruffed lemur (*Varecia variegata*). *American Journal of Primatology*, e23484. https://doi.org/10.1002/ajp.23484