

Article

A comparison of fecal sampling and direct feeding observations for quantifying the diet of a frugivorous primate

Jaya K. MATTHEWS^{a,b,*}, Amanda RIDLEY ^c, Beth A. KAPLIN^{d,e}, and Cyril C. GRUETER^{a,b,c}

^aSchool of Human Sciences, The University of Western Australia, Crawley, WA 6009, Australia, ^bUWA Africa Research & Engagement Centre, The University of Western Australia, Crawley, WA 6009, Australia, ^cCentre for Evolutionary Biology, School of Biological Sciences, The University of Western Australia, Crawley, WA 6009, Australia, ^dCentre of Excellence in Biodiversity and Natural Resource Management, University of Rwanda, Huye, Rwanda, and ^eSchool for the Environment, University of Massachusetts, Boston, MA 02125-3393, USA

*Address correspondence to Jaya K. Matthews. E-mail: jaya.matthews@research.uwa.edu.au.

Handling editor: Baoguo Li

Received on 1 August 2019; accepted on 21 November 2019

Abstract

Both observational and indirect evidence are widely used to determine the diets of wild animals. Direct observations are often assumed to provide the most comprehensive reflection of diet, but many wild animals are logistically challenging to observe. Despite the regular use of observational and indirect methods for inferring diet in wild animals, they have rarely been compared in detail for the same study population. Over 12 months this study assessed the congruence of methods in estimating the diet of a montane community of eastern chimpanzees *Pan troglodytes schweinfurthii* in Nyungwe National Park, Rwanda using observational scan samples and macroscopic fecal inspection. The assessment of the number of food species consumed each month was comparable between methods, but the estimation of the composition of items in the diet differed significantly. Most notably, the fecal samples significantly underestimated the consumption of flowers, and certain fruit species, which based on direct behavioral observations were seasonally consumed at very high rates. Conversely, direct observations underestimated the consumption of leaves and pith in comparison to results present in the fecal samples. These results suggest that combining methods where possible is most useful for accurate monitoring of dietary trends, particularly for species that experience significant seasonal shifts in their diet.

Key words: chimpanzee, comparative methods, dietary analysis, seasonality

Describing the diets of wild animals is a crucial task and central theme in the field of biology, because understanding the dietary ecology of different species provides essential information about habitat requirements, trophic interactions, and food webs (Chapman 1995; Bridgeland et al. 2010; VelezLiendo et al. 2013; Singer et al. 2014). Characterizing the resource base of wild animals can also address questions related to nutritional ecology (Felton

et al. 2009; Birnie-Gauvin et al. 2017), resource partitioning (Wrangham et al. 1998; Pardo et al. 2015), and selective pressures that shape morphology and socio-ecology (Marshall and Wrangham 2007; Chapman et al. 2012). Dietary studies also play a central role in assessing the responses of wild populations to anthropogenic habitat alteration (McLennan 2013; Birnie-Gauvin et al. 2017). This kind of information is especially vital for the conservation efforts of

rare or endangered species, because it helps to identify habitats or food sources that need to be maintained (Ward et al. 2012; Aryal et al. 2016).

Two principal methods for assessing the dietary ecology of wild animal populations are direct observations of feeding activity and analysis of fecal samples (Moreno-Black 1978; Altmann 1991; Martin and Bateson 2007; Gilby et al. 2010; Koirala et al. 2016). Focal animal sampling and instantaneous scan sampling are typical observational methods used to measure activity budgets, time spent feeding, and intake rates (Altmann 1991, 1998; Zuberbühler and Wittig 2011; Kaplin and William 2013). However, these techniques can be subject to observer error, observer bias, and results may be influenced by data recorded predominantly in parts of the habitat in which a species is most observable (McInnis et al. 1983; Shrestha and Wegge 2006; Schneider et al. 2010; Tuytens et al. 2014). Additionally, the processing or handling time of different foods may not be proportional to the amount of that food consumed (Hohmann 2009). For example, in howler monkeys *Alouatta pigra* leaves accounted for 50.6% of time spent feeding, but estimates of dry matter intake of leaves indicated that they accounted for 37% of ingested food (Righini et al. 2017); similarly observations of chimpanzees *Pan troglodytes* were found to overestimate ingested foods (Hladik 1977). Sampling bias may also be introduced by the length of the sampling period and seasonal variation in diet, time of day, and sex and age of the observed individual (Hladik 1977; Altmann 1991, 1998; Rothman et al. 2012).

Direct observations are not always possible since many wild animals are difficult to locate and reliably observe. Researchers may also make ethical decisions, selecting indirect methods to reduce negative impacts (such as disturbance or disease transmission) on the study population (Gruen et al. 2013; Shutt et al. 2014). Therefore, non-invasive methods, such as macroscopic fecal sampling (Tutin and Fernandez 1993; Basabose 2002), microscopic and molecular fecal analysis (Phillips 2011), DNA barcoding of fecal samples (Quéméré et al. 2013; Mallot et al. 2018), and examination of trail signs and feeding remains (Rogers et al. 1990; Doran et al. 2002), can be important for determining animal diets, particularly of shy or at-risk species. However, the choice of indirect methods and their associated biases will vary depending on the study species and the research questions to be answered.

Macroscopic fecal inspection is used to assess diet in a wide range of species (birds: Inger et al. 2006; elephants and antelope: Kos et al. 2012; bears: Koike et al. 2013), including primates (Julliot and Sabatier 1993; Tutin and Fernandez 1993; Doran et al. 2002; Hanya et al. 2003; Surbeck et al. 2009; Phillips and McGrew 2014; Johnson 2015). However, ingested foods have differential rates of digestion, and this approach may result in bias toward items that show greater digestive resistance due to their biochemical and physiological properties (e.g., seeds, hard leaves, or chitinous invertebrate exoskeletons) (Tutin and Fernandez 1993; Mitra and Flynn 2007; Brett et al. 2017). However, it is worth noting that the detection of hard-bodied invertebrates during macroscopic fecal analysis may be influenced by the presence of a functional chitinase gene that assists with the digestion of chitin (Janiak et al. 2017). Furthermore, food classes that leave no recognizable remains (e.g., some leaves, flowers, and soft-bodied invertebrates) may be absent from estimates—these limitations have been well documented in previous studies (McInnis et al. 1983; Putman 1984; Dellinger and Trillmich 1988; Tutin and Fernandez 1993; Su and Lee 2001). Additionally, some items are difficult to identify (e.g., pith and stems), and this may lead to underestimation of these food types in the diet (Tutin

and Fernandez 1993; Su and Lee 2001; Basabose 2002; Parker and Bernard 2006; Phillips and McGrew 2014).

Despite the varying capacities of these approaches to quantify wild animal diets, few studies have made direct intraspecific comparisons of macroscopic fecal sampling and direct feeding observations (reviewed by Nielsen et al. 2018). Research has focused on comparisons of invasive and non-invasive indirect methods (Anthony and Smith 1974; Seefelt and Gillingham 2006; Klare et al. 2011; Weiser and Powell 2011; Bryan 2014) or observational methods (Fragaszy et al. 1992; Margalida et al. 2005; Gilby et al. 2010; Amato et al. 2013). Some studies have directly compared diet composition estimated from observations with macroscopic fecal analysis (McInnis et al. 1983; Mills 1992; Parker and Bernard 2006; Bakaloudis et al. 2012; Phillips and O'Connell 2016), but to date no studies have made direct comparisons using group-level analyses across seasons for chimpanzees (Phillips and McGrew 2014).

Chimpanzees are an ideal species with which to compare the diet profiles generated by these two techniques, since indirect (macroscopic fecal analysis: Tutin and Fernandez 1993; Basabose 2002; McLennan 2013; Phillips and Lancelotti 2014; Carvalho et al. 2015) and direct methods (focal sampling: Wrangham et al. 1996; Potts et al. 2011; scan sampling: Newton-Fisher 1999; Tweheyo et al. 2003) have been employed widely. Determining the breadth and composition of species in the diet using these methods are subject to the above-mentioned biases. Of particular concern is the limited identification and estimation of foods easily digested in fecal samples, and the difficulty observing certain feeding behaviors, which leads to inaccurate estimates. While researchers are often aware of these limitations in general, direct comparisons of methods provide a means for assessing how they might specifically affect results from research on a species or population. Dietary ecology is a particular focus in chimpanzee studies because it can facilitate conservation of this endangered species. Knowledge of potential sources of bias in determining the diet of chimpanzee populations can improve accuracy in identifying important food species and can assist with the protection of those species from anthropogenic influences (Chapman et al. 2000; Felton et al. 2010; Potts 2011).

Comparisons of chimpanzee focal observations and macroscopic fecal inspection in Budongo, Uganda indicated that the proportion of fruits estimated in the diet from both methods were positively correlated, but young leaves were not (Fawcett 2000). The study provided no discussion or explanation as to why this was the case, but it could be argued that the digestibility of young leaves may account for the difference. Research comparing the number of plant or animal items detected from focal observations and macroscopic fecal inspection in Kibale National Park, Uganda concluded that the indirect method was less accurate in describing folivorous and faunivorous diet types (Phillips and McGrew 2013). The difference here was likely due to the difficulties of identifying leaf, pith, or animal fragments in the fecal samples (Tutin and Fernandez 1993; Phillips and McGrew 2013). This research also indicated that when analyzing fecal samples in two subsets, one considering gut passage rates and the other not, the results were similar (Phillips and McGrew 2013). However, the study did not examine the time spent feeding on different food types. An opposite trend was reported in a study on macaques *Macaca cyclopis*, where more vine/shrub species were detected in fecal samples than through observations (Su and Lee 2001); presumably because feeding on those items was difficult to observe.

Seasonal differences in diet could lead to seasonal differences in food digestibility because different foods are consumed, and

therefore, correlations between macroscopic fecal analysis and behavioral observations may differ across seasons. Research spanning an annual cycle that compares the relative proportions of each main food type in the chimpanzee diet between seasons would demonstrate how reliably the two methods correlate in their predictions of chimpanzee diet over time. This is particularly important for species that experience considerable seasonality in the types of food available to them (van Schaik et al. 1993; Hemingway and Bynum 2005), such as the chimpanzee community in the montane forest of Nyungwe National Park, Rwanda (Matthews et al. 2019).

Little research has undertaken detailed and direct comparisons using group-level analyses of dietary data collected from both direct observations and macroscopic fecal analyses between seasons remain unexplored in chimpanzees. Interpretations of diet for any species from observations, macroscopic fecal sampling, or a combination of methods should (if possible), be made with knowledge of the methodological incongruences. With this in mind, the purpose of this study was to 1) highlight any differences in dietary breadth and diversity estimated using direct group-level scans and macroscopic fecal sampling and 2) determine the comparability of diet composition estimated using those two methods across an annual cycle to include seasonal differences. This study does not aim to promote one method over another, but highlight the key differences of each method in order to promote informed methodological choice, accurate interpretations, and cross-site examinations.

Materials and Methods

Study site and subjects

The study was conducted in Nyungwe National Park, Rwanda ($2^{\circ}17' -2^{\circ}50'S$ and $29^{\circ}07' -29^{\circ}26'E$, Figure 1). The 1,019 km² park is located in southwestern Rwanda and connects with Kibira National Park, Burundi. The park is characterized by steep slopes ranging from 1,600 to 2,950 m ASL and contains a mosaic of primary and secondary forest. During the study period (September 2016 to August 2017) annual rainfall was 1,402 mm and mean daily temperature ranged from 11.4°C to 19.6°C (Meteorological station location: $2^{\circ}28'43''S$, $29^{\circ}12'00''E$, 2465 m ASL Nyirambangutse et al. 2017). September to November and January to March were the rainy seasons (monthly rainfall >100 mm), April to August was the major dry season (monthly rainfall <100 mm), and December was also dry (<100 mm), tree fruit production peaked in the major dry season (Matthews et al. 2019). The park has a rich primate diversity, containing at least 13 species of primates, including one species of great ape: the eastern chimpanzee *Pan troglodytes schweinfurthii*. The focal subjects for this study were the Mayebe community of chimpanzees, numbering ~67 free-ranging individuals (18 adult females and 14 adult males individually recognizable, Green et al. 2019), this community receives regular tourists and generally tolerates human presence at >20 m.

Data collection

We collected data from September 2016 to August 2017 for 10 days each month (split into two slots of five consecutive days, with 2 weeks between each sampling period) using 2 methods: instantaneous scan sampling and fecal sampling. Sampling effort occurred on concurrent days throughout the study.

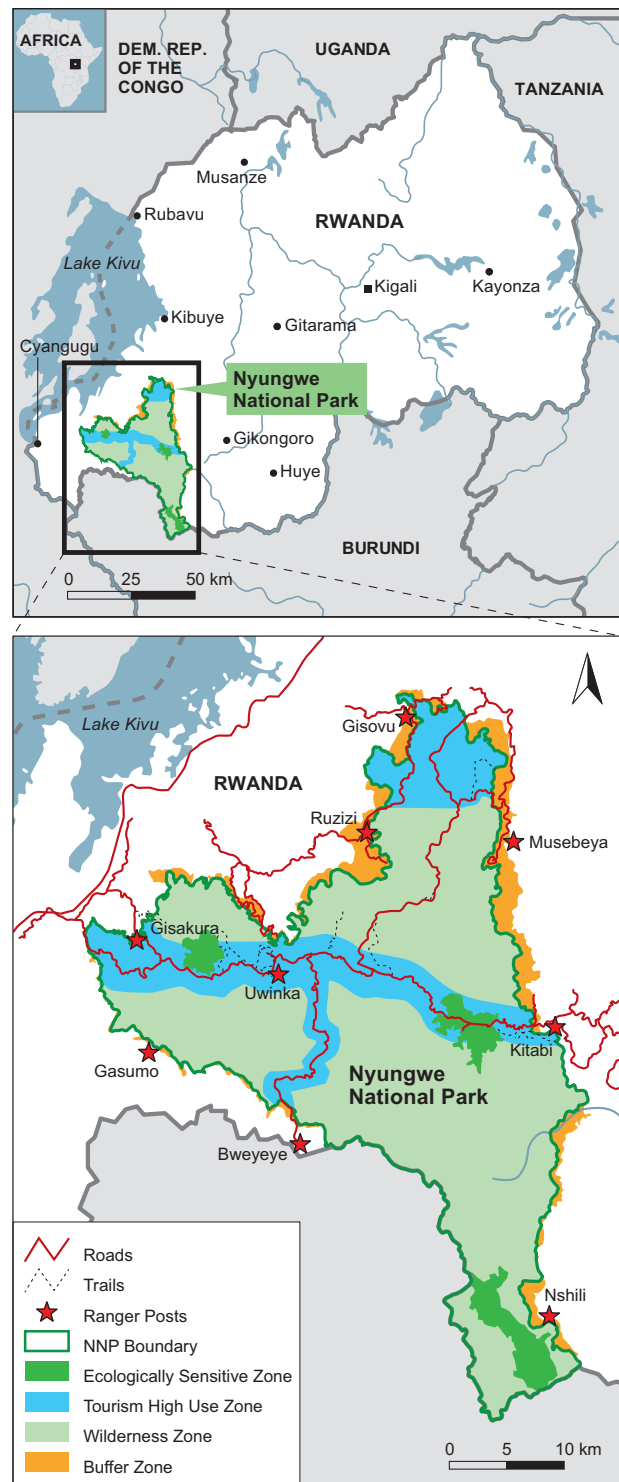


Figure 1. Map of Nyungwe National Park, located in southwestern Rwanda, East Central Africa. Adapted by K. Meisterhans and C. C. Grueter from Nyungwe National Park Management Plan 2012–2021.

Observational data

We collected observational data through instantaneous scan samples (Altmann 1974) taken at 15-min intervals ($n = 1,532$ feeding scans over 12 months, 14.2 ± 8.4 scans per day, 2.5 ± 1.7 individuals feeding per scan). While the terrain, dense vegetation, and large home range precluded the possibility of conducting individual focal

follows, we took care not to resample the same individuals in the group scans by using distinguishing features (e.g., size, hair color, facial features, physical marks, or disfigurements) as a form of identification. We located the chimpanzees at their nests before dawn (when the previous day's nesting sites were known) and followed them throughout the day (for full-day focal group follows) to the evening nest site when possible. Alternatively, we used vocalizations and recently used feeding sites or fresh tracks to locate the chimpanzees. During each group-level scan we noted whether each individual was feeding and if so, we recorded the item (e.g., plant, animal, and mushroom), species, and part consumed (fruit, pith/stem, leaf, flower, bark, and wood). We calculated the composition of the diet as the proportion of scans in which individuals fed on a non-plant item or plant part relative to the number of feeding scans (Matthews et al. 2019). When calculating dietary breadth (number of species consumed) from scan sampling data, we also used feeding remains data associated with the scan samples; if for instance, we could not reliably identify a species fed upon due to poor visibility, we entered the area after the chimpanzees had moved away from the food source, and where possible identified the species from remains (one leaf and one mushroom species unidentified).

Fecal sample data

We collected fresh fecal samples (<24 h old) that were undisturbed by insects opportunistically during the same two 5-day sampling periods as behavioral observations ($n = 323$ samples collected over 12 months of observation). Due to opportunistic collection methods, we were not able to link the fecal samples to a specific age–sex class or known individual. Samples were easily distinguished from other primate feces by size, smell, form, and color. The samples were taken back to camp, stored in plastic zip-lock bags, and analyzed within 5 days of collection. We weighed and then sluiced the entire wet sample in 1 mm mesh sieves (McGrew et al. 2009). We examined each sample macroscopically, and categorized each item (fruit, leaf, pith, flower, animal, or honey), identifying them to species level where possible (two fruit and one leaf species unidentified). We estimated each category visually as a proportion of the total fecal sample volume, to the nearest 5% (Basabose 2002), and counted each fruit seed unless <2 mm in diameter, in which case they were estimated.

All research protocols were reviewed and approved by the University of Western Australia's Animal Ethics Committee (RA/5/15/1070) as well as the governing body of Nyungwe NP, the Rwanda Development Board—Tourism and Conservation Department.

Analytical methods

We used Pearson's correlations to assess the relationship between the number of fruit species, non-fruit plant species (of which leaves and pith are consumed), and total plant species that the chimpanzees fed on during the five consecutive observation day periods and the number recorded in fecal samples during the same 5 days (data did not deviate significantly from a normal distribution). We also used Pearson's correlations to analyze the relationship between dietary diversity and evenness determined from observations and fecal samples. Shannon's Index [$H' = -\sum [P_i \ln P_i]$, where P_i is the proportion of feeding time (or proportion of each fecal sample) accounted for by i th food type] was used to estimate monthly dietary diversity for observational and fecal data (Shannon and Weaver 1964), and Hill's index [$J' = H'/x$, where x is the total number of species sampled] was used to estimate dietary evenness (Hill 1973; Pielou 1974).

For analyses, we categorized honey and insects together as “non-plant.” During fecal analysis, honey was primarily determined by bee (*Apis mellifera* or Meliponini) remains, since bees are only consumed when the chimpanzees are consuming honey, so the detection rate of honey is assumed to be comparable to insects: due to low consumption rates, these could not be analyzed separately. We assessed whether method type (observational or fecal) influenced the proportion of each category (fruit, leaf, pith, flower, and non-plant) in the scan or fecal samples using separate generalized linear models (GLMs) with a binomial error distribution and a logit link function. The response was highly skewed for observations (e.g., fruit consumption was observed 100% of the time in 66% of group feeding scans) and fecal samples (e.g., 72% of fecal samples did not contain flowers), precluding the use of a Poisson distribution. The response was partitioned into a binomial denominator (total possible occurrence of a category was 1) and a numerator (the proportion of each food category present in the sample). We included season (wet or dry months) and interactions between season and method as additional predictors were also included to assess whether there was a seasonal or season * method effect on the estimation of diet. The full model was:

the proportion of a food category in the diet (averaged for each 5–day consecutive sampling period)
 \sim method(observational or fecal) + season(wet or dry)
 + method * season.

To demonstrate trends on a finer scale we also analyzed the data by month using the same method and model as above, but with month as the additional predictor instead of season. Data were analyzed in SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

A comparison of the plant foods consumed for each method type revealed that the number of plant species fed upon during direct foraging observations positively predicted the number of plant species detected through macroscopic fecal inspection (Pearson correlation, $n = 21$ 5-day comparisons, $r = 0.48$, $P < 0.05$; Figure 2A). When considering fruit and non-fruit (e.g., herbaceous) species separately, the number of fruit species consumed was highly correlated between methods, but non-fruits were not, specifically feeding observations detected fewer non-fruit species (fruit: $n = 21$, $r = 0.54$, $P = 0.007$; non-fruit: $n = 21$, $r = 0.34$, $P = 0.11$; Figure 2B,C). Dietary diversity (H') and evenness (J') were not significantly correlated between data collection methods during the 5-day field trips (H' : $n = 10$, $r = 0.16$, $P = 0.61$; J' : $n = 10$, $r = 0.07$, $P = 0.81$).

There was a significant effect of the interaction between season and method on the proportion of fruit, leaves, pith, flowers, and non-plant items detected in the observational scans and fecal samples, with greater similarities in dietary information between method collection types in one season than the other (Table 1). While the model in Table 1 was assessed at a seasonal level, Figures 3–7 and model in Table 2 are presented at the month level to demonstrate precisely where the differences and similarities lie. The proportion of fruits consumed by chimpanzees using the two sampling methods was comparable only during September (wet season), December, and May (dry season, Figure 3 and Table 2). The most substantial differences were apparent during November, January, February, and August (wet season and end of the dry season, Figure 3).

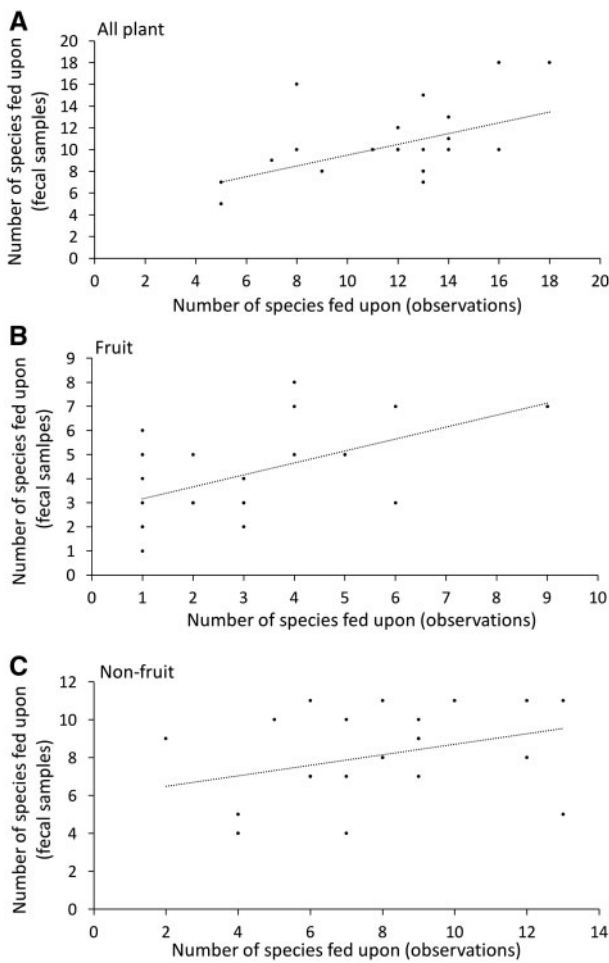


Figure 2. The relationship between the number of **A)** all plant species **B)** fruit species, and **C)** non-fruiting (i.e., herbaceous) species detected in the chimpanzee diet through direct feeding observations and macroscopic fecal samples during bi-monthly field trips from August 2016 to September 2017 in Nyungwe National Park, Rwanda.

In these months, direct foraging observations predicted much higher proportions of fruit in the diet than fecal samples; the reverse trend was apparent during March, April, and June (dry season and end of the wet season, [Figure 3](#)).

Pith was consistently estimated at significantly higher proportions in the fecal samples than direct observations ([Figure 4](#) and [Table 2](#)); similarly, leaves were estimated at higher rates in the fecal samples during 9 out of the 12 months ([Figure 5](#) and [Table 2](#)). The opposite trend was observed for flowers: they were estimated at higher proportions during direct observations than fecal samples in 6 out of 12 months ([Figure 6](#)). Meat was present in fecal samples during 4 months of the year (wet and dry season), but no hunting or meat-eating was detected during observational sampling. Honey and insects were detected at low rates using both methods, and the proportions of these items in the chimpanzee diet appeared to be similar only during October (wet season, [Figure 7](#)). The data indicated that in 5 months (mostly wet season) these items were more prevalent in the fecal samples, while in 4 months (mostly dry season) they were more prevalent in observations. Overall low detection rates precluded a month by month statistical comparison and made a clear trend difficult to identify ([Figure 7](#)).

Discussion

Our comparison of the two techniques of dietary data collection over the time period in the same study population demonstrated that there was a highly significant difference in the estimation of dietary diversity and seasonal dietary composition of fruit, leaf, pith, flowers, and non-plant items using observational scans and macroscopic fecal inspection. However, differences were not consistent throughout the study, and during some seasons, and months, the two methods generated comparable results, while others differed markedly. Highly obvious disparities were evident in the low estimation of leaves and pith in the observational data, and low estimation of flowers in the fecal samples. The study also indicated that each method detected comparable numbers of fruit, but not non-fruit plant items such as leaves and pith in the monthly diet.

Studies comparing dietary analysis methods on a range of species have also highlighted disparities among techniques in determining dietary breadth ([Klare et al. 2011](#); [Bakaloudis et al. 2012](#)) and composition ([McInnis et al. 1983](#); [Seefelt and Gillingham 2006](#); [van Lieverloo et al. 2009](#); [Wam and Hjeljord 2010](#)). For example, pellet analysis of double-crested cormorant *Phalacrocorax auritus* diets undervalued the importance of the prey species alewife *Alosa pseudoharengus* compared with other methods ([Seefelt and Gillingham 2006](#)). [Seefelt and Gillingham \(2006\)](#) highlighted the significance of this oversight, since alewife is an important prey species for the cormorant diet, with an important link between cormorant reproductive success and alewife population dynamics ([Weseloh and Ewins 1994](#); [Wires et al. 2001](#)). During November (wet season), observations of Nyungwe chimpanzees indicated that they fed almost exclusively on the fruit of *Syzygium guineense* ([Matthews et al. 2019](#)). However, seed handling behavior (wadging in this instance, i.e., extracting juice and nutrients from fruits while spitting the skin, fiber, and seeds out in a ball) influenced what remained of *S. guineense* in the fecal samples, underestimating the importance of this fruit species in the diet over the same period considerably.

The chimpanzees are an important seed disperser of *S. guineense* ([Gross-Camp et al. 2009](#)), and analyzing fecal samples alone would not reveal this relationship. Likewise, in January, February, and August, the dominant species consumed (*Ficus sur* and *Podocarpus latifolius*) were both wadged, and the fecal samples yielded lower proportions of fruits than the observations ([Matthews et al. 2019](#)). We explored the alternative explanation that fruit estimates in November, January, February, and August were influenced by a lack of correlation between seed size and fruit size of the dominant plant species consumed. While this does not appear to have had a substantial impact on our results in these months, detailed data on seed and fruit size are not available for all species consumed, and therefore this may have been a source of bias in other months. Future studies estimating fruit consumption from fecal samples should consider the relationship between seed and fruit size when making their estimations.

We observed very high rates of flower consumption toward the end of the wet season and into the dry season (March, April, and June), yet flowers were detected at meager rates or not at all in the fecal samples. At the end of the wet season (March) this difference was so significant that >70% of the observed diet was comprised of flowers, while 0% of flower remains were detected in fecal samples. If we had relied on fecal samples alone we would be unaware of the large disparity in the two methods, and would not have noticed this interesting dietary behavior given that flowers are not typically an

Table 1. GLM analysis of the effect of method (observational scans and macroscopic fecal sampling) and season on the proportion of fruit, pith, leaf, flower, and non-plant items (honey and insects) in the chimpanzee diet over a 12-month period (2016–2017)

Food type	Predictors	χ^2	P-value	Effect size \pm SE	95% CI
Fruit	Method	35	<0.001	Fec: -0.36 ± 0.06 (Obs: 0 _a)	-0.48, -0.24
	Season	23	<0.001	Dry: 0.29 ± 0.06 (Wet: 0 _a)	0.17, 0.41
	Method * Season	83	<0.001	Fec*Dry: -0.08 ± 0.08	-0.26, 0.08
				Obs*Dry: -0.02 ± 0.09	-0.19, 0.15
Pith	Method	241	<0.001	Fec: 2.11 ± 0.13 (Obs: 0 _a)	1.84, 2.37
	Season	18	<0.001	Dry: 0.41 ± 0.09 (Wet: 0 _a)	-0.61, -0.22
	Method * Season	272	<0.001	Fec*Dry: 2.19 ± 0.23	1.73, 2.65
				Obs*Dry: 0.69 ± 0.26	0.17, 1.21
Fec*Wet: 2.80 ± 0.23 (Obs*Wet: 0 _a)				2.35, 3.26	
Leaf	Method	53	<0.001	Fec: 0.65 ± 0.08 (Obs: 0 _a)	0.47, 0.82
	Season	8	<0.05	Dry: -0.16 ± 0.08 (Wet: 0 _a)	0.04, 0.26
	Method * Season	56	<0.001	Fec*Dry: 0.50 ± 0.12	0.25, 0.75
				Obs*Dry: -0.12 ± 0.14	-0.39, 0.16
Flower	Method	235	<0.001	Fec: -2.92 ± 0.19 (Obs: 0 _a)	-3.29, -2.55
	Season	10	0.002	Dry: 0.34 ± 0.10 (Wet: 0 _a)	0.55, 0.12
	Method * Season	245	<0.001	Fec*Dry: -2.98 ± 0.22	-3.43, -2.54
				Obs*Dry: -0.42 ± 0.11	-0.64, -0.19
Non-plant	Method	8	0.002	Fec: -0.87 ± 0.25 (Obs: 0 _a)	-0.93, -0.53
	Season	29	<0.001	Dry: -1.79 ± 0.33 (Wet: 0 _a)	-2.45, -1.15
	Method * Season	30	<0.001	Fec*Dry: -2.54 ± 0.60	-3.73, -2.35
				Obs*Dry: -1.56 ± 0.39	-2.33, -1.77
				Fec*Wet: -0.30 ± 0.27 (Obs*Wet: 0 _a)	-1.34, -0.24

0_a is set to zero because this category level is the reference category. Fec, fecal sampling; Obs, observational sampling.

Table 2. GLM analysis of the effect of method (observational scans and macroscopic fecal sampling) and month on the proportion of fruit, pith, leaves, and flowers in the chimpanzee diet over a 12-month period (2016–2017)

Predictors	Fruit:			Pith:			Leaf:			Flower:						
	χ^2	Effect \pm SE	95% CI	χ^2	Effect \pm SE	95% CI	χ^2	Effect \pm SE	95% CI	χ^2	Effect \pm SE	95% CI				
Method	38			247			54			266						
		Fec	-0.39 ± 0.06	$-0.52, -0.27$		2.15 ± 0.13	$1.89, 2.42$		-0.67 ± 0.09	$0.49, 0.85$		-3.24 ± 0.19	$-3.63, -2.85$			
		Obs	0 _a		0 _a		0 _a		0 _a		0 _a					
Met*Mon	509	See Figure 3			244	See Figure 4			243	See Figure 5			547	See Figure 6		
Month	329			121			129			287						
		Sep	0.45 ± 0.18	$-0.03, 0.95$		-0.58 ± 0.24	$-1.06, -0.10$		-0.71 ± 0.23	$-1.16, -0.26$		-	-			
		Oct	0.53 ± 0.15	$0.24, 0.81$		-1.08 ± 0.21	$-1.51, -0.66$		-0.43 ± 0.17	$-0.76, -0.09$		-	-			
		Nov	0.42 ± 0.14	$0.14, 0.70$		-0.74 ± 0.20	$-1.13, -0.35$		-0.90 ± 0.18	$-1.26, -0.83$		0 _a				
		Dec	0 _a		0 _a		0 _a		0 _a			-	-			
		Jan	0.81 ± 0.15	$0.52, 1.10$		-0.52 ± 0.19	$-0.89, -0.14$		-1.14 ± 0.20	$-1.50, -0.73$		-	-			
		Feb	0.68 ± 0.15	$0.39, 0.97$		-0.84 ± 0.20	$-1.24, -0.43$		-1.19 ± 0.20	$-1.58, -0.79$		1.68 ± 0.36	$0.97, 2.01$			
		Mar	-0.69 ± 0.15	$-0.97, -0.40$		-0.71 ± 0.20	$-1.10, -0.32$		-0.58 ± 0.18	$-0.93, -0.24$		3.61 ± 0.35	$2.92, 3.94$			
		Apr	0.96 ± 0.15	$0.66, 1.25$		-0.91 ± 0.20	$-1.32, -0.51$		-0.71 ± 0.18	$-1.06, -0.36$		2.99 ± 0.35	$2.31, 3.28$			
		May	-0.18 ± 0.14	$-0.46, 0.09$		-1.87 ± 0.26	$-2.39, -1.34$		-0.90 ± 0.19	$-1.27, -0.53$		1.48 ± 0.36	$0.75, 1.82$			
		Jun	0.79 ± 0.15	$0.51, 1.09$		-2.04 ± 0.28	$-2.59, -1.50$		-1.69 ± 0.23	$-2.15, -1.23$		2.28 ± 0.35	$1.58, 2.67$			
		Jul	1.12 ± 0.15	$0.81, 1.41$		-1.61 ± 0.23	$-2.11, -1.13$		-1.40 ± 0.21	$-1.82, -0.98$		0.84 ± 0.38	$0.76, 1.62$			
		Aug	1.63 ± 0.16	$1.30, 1.96$		-1.73 ± 0.25	$-1.99, -0.98$		-2.02 ± 0.26	$-2.54, -1.51$		0.74 ± 0.38	$-0.04, 1.52$			

0_a is set to zero because this category level is the reference category.

important food item in chimpanzee diets (reviewed in Watts et al. 2012). This result supports previous suggestions that macroscopic fecal sampling results in the underrepresentation of foods that are easily broken down during digestion (Tutin and Fernandez 1993), compared with foods such as mature leaves that are high in cellulose and dietary fiber and are more difficult to digest.

On the other hand, during some months macroscopic fecal inspection detected honey and insects in higher quantities than

observations. Underrepresentation of rarer items such as insects by observations compared with other methods of dietary estimation has also been noted in previous studies of primates (Moreno-Black 1978; Tutin and Fernandez 1993; Su and Lee 2001; Radespiel et al. 2006), and is evidently a methodological shortfall of studies relying solely on observations. It is also possible that the 15-min scan interval used in this study could have omitted shorter feeding bouts that included insect feeding. However, there were also months in which

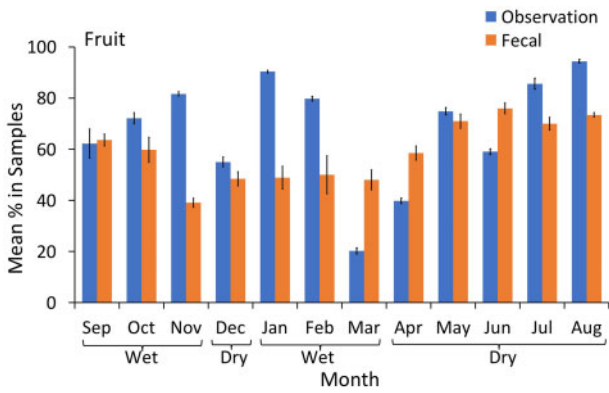


Figure 3. Mean percentage of fruit (with 95% CI) in the chimpanzee diet over 12 months of continuous sampling (2016–2017), using observational scans and macroscopic fecal sampling.

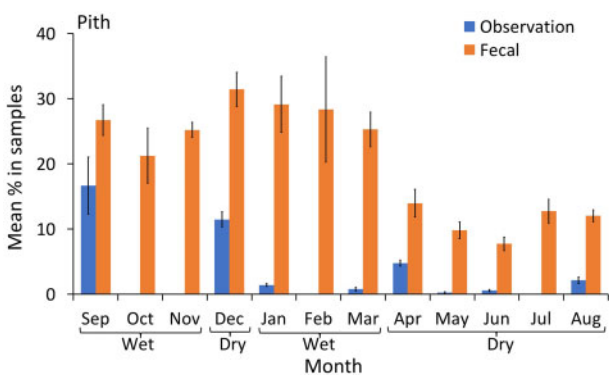


Figure 4. Mean percentage of pith (with 95% CI) in the chimpanzee diet over 12 months of continuous sampling (2016–2017), using observational scans and macroscopic fecal sampling.

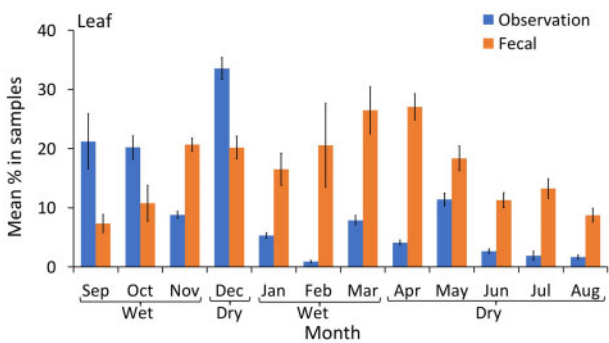


Figure 5. Mean percentage of leaves (with 95% CI) in the chimpanzee diet over 12 months of continuous sampling (2016–2017), using observational scans and macroscopic fecal sampling.

observations detected honey and insect consumption while fecal samples did not, suggesting that utilizing either method alone would result in biased detection rates.

The other obvious disparity between methods was the high proportion of leaves and pith estimated in the fecal samples during a majority of months compared with direct feeding observations. This overestimation is likely due to recognizable fibrous strands of pith and stems remaining in the samples while flowers, fruit pulp, and leaves were digested more easily, further indicating that the

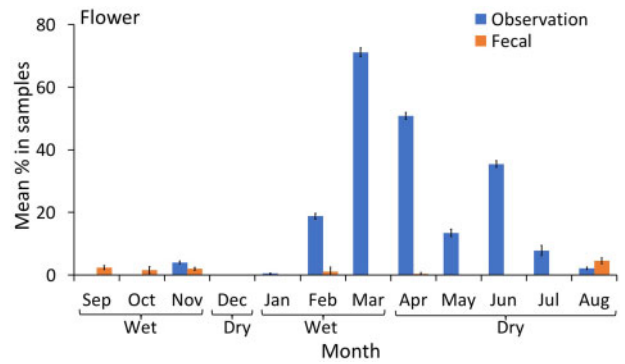


Figure 6. Mean percentage of flowers (with 95% CI) in the chimpanzee diet over 12 months of continuous sampling (2016–2017), using observational scans and macroscopic fecal sampling.

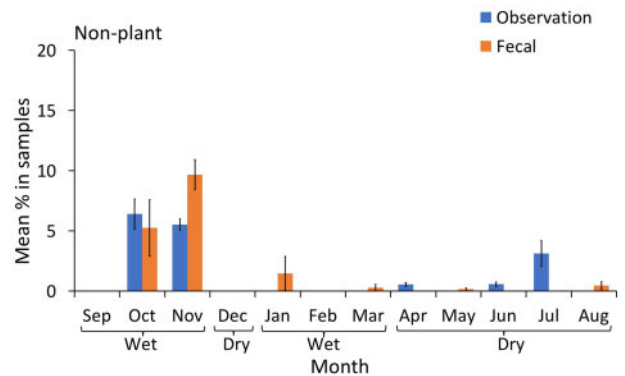


Figure 7. Mean percentage of insects and honey (with 95% CI) in the chimpanzee diet over 12 months of continuous sampling (2016–2017), using observational scans and macroscopic fecal sampling.

biochemical and physical properties of food items, as well as their digestibility, can influence estimates of dietary composition from macroscopic fecal sampling. Another source of bias highlighted by Bakaloudis et al. (2012) suggested that the bias in diet determined by observations of the long-legged buzzard *Buteo rufinus* compared with other methods may in part have stemmed from their selection of easily accessible nests for viewing and the resultant environmental homogeneity. Although we did not select specific areas for viewing the chimpanzees (as in the above study), certain areas and behaviors were more easily visible than others (e.g., tree-feeding versus ground-feeding); this may have similarly biased our observational results as non-fruit plants such as herbaceous vegetation were typically at ground-level. Additionally, estimates of leaves and pith in the fecal samples from February to June (end of wet season/beginning of the dry season) may be inflated due to underestimation of flowers or particular fruiting species during the same period.

Passage rates of foods consumed by the chimpanzees in the analysis of fecal samples may explain some of the discrepancies between sampling methods in this study. Mean gut retention time for chimpanzees in captivity is estimated to be 31–48 h (Milton and Demment 1988; Lambert 2002); estimating passage rates for chimpanzees in the wild is much more difficult due to varied diets and repeated consumption of the same foods over the course of a day, as well as consecutive fecal samples containing the same species (Phillips and McGrew 2013). In this study, the fecal samples collected on day 1 of each 5-day field trip would have contained foods

that were consumed before observations conducted on day 1. This could have contributed to the divergence in diet estimations when averaging across each sampling period. However, Phillips and McGrew (2013) demonstrated that the results were similar when considering gut passage rates during comparisons of fecal sample analysis and direct observations. Future comparative studies may account for this potential bias by offsetting observations with fecal sampling to account for the lag between observations of food consumption and when food would likely appear in the fecal samples. In addition, the lack of correlation between methods used in this study demonstrates that the proportion of time that chimpanzees were observed feeding on particular species does not translate to the amount of food consumed, and thus the inclusion of information such as food intake from bite/unit consumption rates in future comparisons may produce a better correlation with fecal samples (Rothman et al. 2012).

When monthly dietary breadth was compared, the number of fruit species recorded in the diet using both methods was correlated, but the number of non-fruit (e.g., herbaceous) plant species was not. In contrast to our findings, there was no correlation between the mean number of species observed to be fed upon by chimpanzees and those detected in fecal samples in Budongo Forest, Uganda (Fawcett 2000). The reason for this difference is unclear, but it can be argued that in our study the correlation of fruiting species detected by both methods is likely due to chimpanzees being more observable while feeding in fruiting trees, and fruit seeds being easily identified in fecal samples (Tutin and Fernandez 1993). The effect of visibility on observations is likely also the reason that dietary diversity (H') and evenness (J') were both significantly higher in fecal samples compared with direct observation. This reveals that the choice of method can significantly influence the outcome of diet analyses, and this may lead to different conclusions regarding the ecology and habitat requirements of a species (Klare et al. 2011).

Data analyzed in this paper confirms that the two data collection types we used differed significantly in their estimation of dietary composition. In the present study on the Nyungwe chimpanzees, direct observations underestimated dietary diversity and the amount of leaf and pith plant items in the diet; while reliance on fecal sampling significantly underestimated the consumption of flowers. Both techniques have significant biases and flaws and without direct comparisons such as the one undertaken in this study, predicting the extent of the biases is challenging, particularly for species with a broad diet of items that vary widely in digestibility. Our results suggest that when addressing questions related to dietary breadth and diversity, a combination of both observational and fecal sampling methods will provide the most comprehensive description of the diet. While our results indicate that both methods used in this study could not provide a knowingly accurate account of diet composition, this is a common methodological issue with field studies that is not often addressed. Using multiple methods (e.g., macroscopic and microscopic analysis of fecal samples as well as observations, camera traps, and if possible, bite/unit consumption counts) to collect data concurrently can demonstrate where bias may be present, thus improving the interpretability of results as different methods can result in different conclusions about a species' ecological and habitat needs. Other studies have similarly concluded that a combination of methods is the most effective approach (Parker and Bernard 2006; Bakaloudis et al. 2012). A study comparing methods of dietary analysis of giraffes *Giraffa camelopardalis* found congruency in their results from direct observations and fecal analysis in identifying seasonal changes in the top food species, yet the authors still

recommended that a combination of methods should be used (Parker and Bernard 2006). These findings suggest that features of the animal's foraging behavior and its environment (e.g., selective feeder, diurnal, and open habitat) influence the suitability and accuracy of different methods. However, we also highlight that when selecting appropriate methodological approaches, it is important to consider how the chosen methods relate to the research questions and goals, the feasibility of applying within the system of interest, and any ethical and conservation-related issues.

While our discussion relates mainly to macroscopic fecal sampling we also highlight the contribution that microscopic sampling techniques such as DNA barcoding or pollen analysis provide in studies assessing the dietary diversity, composition, and seasonal shifts of a range of species (Valentini et al. 2009; Scanlon and Petit 2013; Garnick et al. 2018; Komura et al. 2018; Robeson et al. 2018), particularly as these methods can deliver precision in species identification (Quéméré et al. 2013; Hamad et al. 2014). It is important to note that the DNA barcoding marker used to analyze plant diet can impact which species are identified (Bradley et al. 2007; Mallot et al. 2018), and when identifying vertebrates in the diet through DNA analysis there is the possibility that vertebrates in the local environment contaminated the feces (Hofreiter et al. 2010). Evidently, an integrative approach that utilizes macroscopic fecal analyses and/or behavioral observations to confirm DNA results would be the most effective (Ortmann et al. 2006; Bradley et al. 2007).

The differences in diet composition and diversity estimated by the two sampling methods used in this study highlight some of the key considerations to be made when using one single method to calculate wild animal diets. Future studies should empirically assess whether differences exist among methods used to determine diet in a range of species. Of particular interest are animals in highly seasonal environments where differences may change over an annual cycle, and rare or endangered species, since practical conservation efforts often rely on accurate knowledge of a species' ecology.

Acknowledgments

We thank the government of Rwanda for allowing us to work in Nyungwe National Park and the Rwanda Development Board, specifically Innocent Ndikubwimana and Kambogo Ildephonse for facilitating the research. Field work was made possible through assistance and dedication from Fidele Muhayyezu and the chimpanzee trackers, and Samantha Green and Bradley Smith for their collaboration in the field. We also thank Göran Wallin for providing climate data. We are grateful to the anonymous reviewers who provided feedback on this manuscript, which has improved the clarity and quality of this work.

Funding

Funding was provided by the University of Western Australia, UWA Postgraduate Student Association and Basler Stiftung für Biologische Forschung (Switzerland).

References

- Altmann J, 1974. Observational study of behavior: sampling methods. *Behaviour* 49:227–266.
- Altmann SA, 1991. Diets of yearling female primates *Papio cynocephalus* predict lifetime fitness. *Proc Natl Acad Sci U S A* 88:420–423.
- Altmann SA, 1998. *Foraging for Survival: Yearling Baboons in Africa*. Chicago (IL): The University of Chicago Press.

- Amato KR, Van Belle S, Wilkinson B, 2013. A comparison of scan and focal sampling for the description of wild primate activity, diet and intragroup spatial relationships. *Folia Primatol* 84: 87–101.
- Anthony RG, Smith NS, 1974. Comparison of rumen and fecal analysis to describe deer diets. *J Wildl Manag* 38:535–540.
- Aryal A, Lamsal RP, Ji W, Raubenheimer D, 2016. Are there sufficient prey and protected areas in Nepal to sustain an increasing tiger population?. *Ethol Ecol Evol* 28:1–4.
- Bakaloudis DE, Iezekiel S, Vlachos CG, Bontzorlos VA, Papakosta M et al., 2012. Assessing bias in diet methods for the long-legged buzzard *Buteo rufinus*. *J Arid Environ* 77:59–65.
- Basabose AK, 2002. Diet composition of chimpanzees inhabiting the montane forest of Kahuzi, Democratic Republic of Congo. *Am J Primatol* 58:1–21.
- Birnie-Gauvin K, Peiman KS, Raubenheimer D, Cooke SJ, 2017. Nutritional physiology and ecology of wildlife in a changing world. *Conserv Physiol* 5: 1–18.
- Bradley BJ, Stiller M, Doran-Sheehy DM, Harris T, Chapman CA et al., 2007. Plant DNA sequences from feces: potential means for assessing diets of wild primates. *Am J Primatol* 69: 699–705.
- Brett MT, Bunn SE, Chandra S, Galloway AWE, Guo F et al., 2017. How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshw Biol* 62:833–853.
- Bridgeland WT, Beier P, Kolb T, Whitham TG, 2010. A conditional trophic cascade: birds benefit faster growing trees with strong links between predators and plants. *Ecology* 91:73–84.
- Bryan A, 2014. Identifying bearded and ringed seal diet: a comparison of stomach contents, stable isotopes, fatty acids, and fecal DNA [masters thesis]. [Fairbanks (USA)]: University of Alaska Fairbanks.
- Carvalho JS, Vicente L, Marques TA, 2015. Chimpanzee *Pan troglodytes* versus diet composition and food availability in a human-modified landscape at Lagoas de Cufada Natural Park, Guinea-Bissau. *Int J Primatol* 36: 802–822.
- Chapman CA, 1995. Primate seed dispersal: coevolution and conservation implications. *Evol Anthropol Issues News Rev* 4:74–82.
- Chapman CA, Balcomb SR, Gillespie TR, Skorupa JP, Struhsaker TT, 2000. Long-term effects of logging on African primate communities: a 28-year comparison from Kibale National Park, Uganda. *Conserv Biol* 14:207–217.
- Chapman CA, Rothman JM, Lambert JE, 2012. Food as a selective force in primates. In: Mitani JC, Call J, Kappeler PM, Palmobit J, Silk JB, editors. *The Evolution of Primate Societies*. Chicago (IL): The University of Chicago Press, 149–168.
- Dellinger T, Trillmich F, 1988. Estimating diet composition from scat analysis in otariid seals (*Otariidae*): is it reliable? *Can J Zool* 66:1865–1870.
- Doran DM, McNeilage A, Greer D, Bocian C, Mehlman P et al., 2002. Western lowland gorilla diet and resource availability: new evidence, cross-site comparisons, and reflections on indirect sampling methods. *Am J Primatol* 58: 910–116.
- Fawcett K, 2000. Female relationships and food availability in a forest community of chimpanzees [PhD thesis]. [Edinburgh (UK)]: University of Edinburgh.
- Felton AM, Felton A, Foley WJ, Lindenmayer DB, 2010. The role of timber tree species in the nutritional ecology of spider monkeys in a certified logging concession, Bolivia. *Forest Ecol Manag* 259:1642–1649.
- Felton AM, Felton A, Lindenmayer DB, Foley WJ, 2009. Nutritional goals of wild primates. *Funct Ecol* 23:70–78.
- Fragaszy DM, Boinski S, Whipple J, 1992. Behavioral sampling in the field: comparison of individual and group sampling methods. *Am J Primatol* 26: 259–275.
- Garnick S, Barboza PS, Walker JW, 2018. Assessment of animal-based methods used for estimating and monitoring rangeland herbivore diet composition. *Rangeland Ecol Manag* 71: 449–457.
- Gilby IC, Pokempner AA, Wrangham RW, 2010. A direct comparison of scan and focal sampling methods for measuring wild chimpanzee feeding behaviour. *Folia Primatol* 81:254–264.
- Green SJ, Boruff BJ, Grueter CC, 2019. From ridge tops to ravines: landscape drivers of chimpanzee ranging patterns. *bioRxiv* <https://doi.org/10.1101/795393>.
- Gross-Camp ND, Masozera M, Kaplin BA, 2009. Chimpanzee seed dispersal quantity in a tropical montane forest of Rwanda. *Am J Primatol* 71: 901–911.
- Gruen L, Fultz A, Pruetz J, 2013. Ethical issues in African great ape field studies. *ILAR J* 54:24–32.
- Hamad I, Delaporte E, Raoult D, Bittar F, 2014. Detection of termites and other insects consumed by African great apes using molecular fecal analysis. *Sci Rep* 4:1–8.
- Hanya G, Noma N, Agetsuma N, 2003. Altitudinal and seasonal variations in the diet of Japanese macaques in Yakushima. *Primates* 44:51–59.
- Hemingway CA, Bynum N, 2005. The influence of seasonality on primate diet and ranging. In: Brockman DK, van Schaik CP, editors. *Seasonality in Primates: Studies of Living and Extinct Human and Non-Human Primates*. Cambridge (UK): Cambridge University Press, 57–104.
- Hill MO, 1973. Diversity and evenness: a unifying notation and its consequences. *Ecology* 54:427–432.
- Hladik CM, 1977. Chimpanzees of Gabon and chimpanzees of Gombe: some comparative data on diet. In: Clutton-Brock TH, editor. *Primate Ecology: Studies of Feeding and Ranging Behavior in Lemurs, Monkeys and Apes*. London (UK): Academic Press, 481–501.
- Hofreiter M, Kreuz E, Eriksson J, Schubert G, Hohmann G, 2010. Vertebrate DNA in fecal samples from bonobos and gorillas: evidence for meat consumption or artefact? *PLoS ONE* 5:e9419.
- Hohmann G, 2009. The diets of non-human primates: frugivory, food processing, and food sharing. In: Hublin JJ, Richards MP, editors. *The Evolution of Hominin Diets: Integrating Approaches to the Study of Palaeolithic Subsistence*. Dordrecht (Netherlands): Springer, 1–14.
- Inger R, Ruxton GD, Newton J, Colhoun K, Robinson JA et al., 2006. Temporal and intrapopulation variation in prey choice of wintering geese determined by stable isotope analysis. *J Anim Ecol* 75:1190–1200.
- Janiak MC, Chaney ME, Tosi AJ, 2017. Evolution of acidic mammalian chitinase genes (CHIA) is related to body mass and insectivory in primates. *Mol Biol Evol* 35:607–622.
- Johnson C, 2015. The feeding and movement ecology of yellow baboons *Papio cynocephalus* in a primate rich habitat: The Issa Valley of Western Tanzania [PhD thesis]. [Wales (UK)]: Swansea University.
- Julliot C, Sabatier D, 1993. Diet of the red howler monkey *Alouatta seniculus* in French Guiana. *Int J Primatol* 14:527–550.
- Kaplin BA, William A, 2013. Behaviour within groups. In: Sterling EJ, Bynum N, Blair ME, editors. *Primate Ecology and Conservation: A Handbook of Techniques*. Oxford (UK): Oxford University Press, 58–78.
- Klare U, Kamler JF, Macdonald DW, 2011. A comparison and critique of different scat-analysis methods for determining carnivore diet. *Mammal Rev* 41:294–312.
- Koike S, Nakashita R, Naganawa K, Koyama M, Tamura A, 2013. Changes in diet of a small, isolated bear population over time. *J Mammal* 94: 361–368.
- Koirala RK, Raubenheimer D, Aryal A, Pathak ML, Ji W, 2016. Feeding preferences of the Asian elephant *Elephas maximus* in Nepal. *BMC Ecol* 16: 1–9.
- Komura T, Ando H, Horikoshi K, Suzuki H, Isagi Y, 2018. DNA barcoding reveals seasonal shifts in diet and consumption of deep-sea fishes in wedge-tailed shearwaters. *PLoS ONE* 13:e0195385.
- Kos M, Hoetmer AJ, Pretorius Y, de Boer WF, de Knegt H et al., 2012. Seasonal diet changes in elephant and impala in mopane woodland. *Eur J Wildl Res* 58:279–287.
- Lambert JE, 2002. Digestive retention times in forest guenons (*Cercopithecus* spp.) with reference to chimpanzees *Pan troglodytes*. *Int J Primatol* 23: 1169–1185.
- Mallot EK, Garber PA, Malhi RS, 2018. *trnL* outperforms *rbcL* as a DNA metabarcoding marker when compared with the observed plant component of the diet of wild white-faced capuchins (*Cebus capucinus*, Primates). *PLoS ONE* 13:e0199556.
- Margalida A, Bertran J, Boudet J, 2005. Assessing the diet of nestling bearded vultures: a comparison between direct observation methods. *J Field Ornithol* 76:40–45.

- Marshall AJ, Wrangham RW, 2007. Evolutionary consequences of fallback foods. *Int J Primatol* 28:1219–1235.
- Martin P, Bateson PPG, 2007. *Measuring Behaviour: An Introductory Guide*. Cambridge (UK): Cambridge University Press.
- Matthews JK, Ridley A, Niyigaba P, Kaplin BA, Grueter CC, 2019. Chimpanzee feeding ecology and fallback food use in the montane forest of Nyungwe National Park, Rwanda. *Am J Primatol* 81:e22971.
- McGrew WC, Marchant LF, Phillips CA, 2009. Standardised protocol for primate faecal analysis. *Primates* 50:363–366.
- McInnis ML, Vavra M, Krueger WC, 1983. A comparison of four methods used to determine the diets of large herbivores. *J Range Manag* 36:302–306.
- McLennan MR, 2013. Diet and feeding ecology of chimpanzees *Pan troglodytes* in Bulindi, Uganda: foraging strategies at the forest-farm interface. *Int J Primatol* 34:585–614.
- Mills MGL, 1992. A comparison of methods used to study food habits of large African carnivores. In: McCullough DR, Barrett RH, editors. *Wildlife 2001: Populations*. London (UK): Elsevier, 1112–1124.
- Milton K, Demment MW, 1988. Digestion and passage kinetics of chimpanzees fed high and low fiber diets and comparison with human data. *J Nutr* 118:1082–1088.
- Mitra A, Flynn K, 2007. Importance of interactions between food quality, quantity, and gut transit time on consumer feeding, growth, and trophic dynamics. *Am Nat* 169:632–646.
- Moreno-Black G, 1978. The use of scat samples in primate diet analysis. *Primates* 19:215–221.
- Newton-Fisher NE, 1999. The diet of chimpanzees in the Budongo Forest Reserve, Uganda. *Afr J Ecol* 37:344–354.
- Nielsen JM, Clare EL, Hayden B, Brett MT, Kratina P, 2018. Diet tracing in ecology: method comparison and selection. *Methods Ecol Evol* 9:278–291.
- Nyirambangutse B, Zibera E, Uwizeye FK, Nsabimana D, Bizuru E et al., 2017. Carbon stocks and dynamics at different successional stages in an Afromontane tropical forest. *Biogeosciences* 14:1285–1303.
- Ortmann S, Bradley BJ, Stolter C, Ganzhorn JU, 2006. Estimating the quality and composition of wild animal diets: a critical survey of methods. In: Hohmann G, Robbins MM, Boesch C, editors. *Feeding Ecology in Apes and Other Primates: Ecological, Physical and Behavioral Aspects*. Cambridge (UK): Cambridge University Press, 397–420.
- Pardo SA, Burgess KB, Teixeira D, Bennett MB, 2015. Local-scale resource partitioning by stingrays on an intertidal flat. *Mar Ecol Prog Ser* 533:205–218.
- Parker DM, Bernard RTF, 2006. A comparison of two diet analysis techniques for a browsing megaherbivore. *J Wildl Manag* 70:1477–1480.
- Phillips CA, 2011. Chimpanzee diet: analyses at macroscopic, microscopic and molecular [PhD thesis]. [Cambridge (UK)]: University of Cambridge.
- Phillips CA, Lancelotti C, 2014. Chimpanzee diet: phytolith analysis of feces. *Am J Primatol* 76:757–773.
- Phillips CA, McGrew WC, 2013. Identifying species in chimpanzee *Pan troglodytes* feces: a methodological lost cause? *Int J Primatol* 34:792–807.
- Phillips CA, McGrew WC, 2014. Macroscopic inspection of ape feces: what's in a quantification method? *Am J Primatol* 76:539–550.
- Phillips CA, O'Connell TC, 2016. Faecal carbon and nitrogen isotopic analysis as an indicator of diet in Kanyawara chimpanzees, Kibale National Park, Uganda. *Am J Phys Anthropol* 161:685.
- Pielou EC, 1974. *Population and Community Ecology: Principles and Methods*. New York (NY): Gordon and Breach.
- Potts KB, 2011. The long-term impact of timber harvesting on the resource base of chimpanzees in Kibale National Park, Uganda. *Biotropica* 43:256–264.
- Potts KB, Watts DP, Wrangham RW, 2011. Comparative feeding ecology of two communities of chimpanzees *Pan troglodytes* in Kibale National Park, Uganda. *Int J Primatol* 32:669–690.
- Putman RJ, 1984. Facts from faeces. *Mammal Rev* 14:79–97.
- Quéméré E, Hibert F, Miquel C, Lhuillier E, Rasolondraibe E et al., 2013. A DNA metabarcoding study of a primate dietary diversity and plasticity across its entire fragmented range. *PLoS ONE* 8:e58971.
- Radespiel U, Reimann W, Rahelinirina M, Zimmermann E, 2006. Feeding ecology of sympatric mouse lemur species in northwestern Madagascar. *Int J Primatol* 27:311–321.
- Righini N, Garber PA, Rothman JM, 2017. The effects of plant nutritional chemistry on food selection of Mexican black howler monkeys *Alouatta pigra*: the role of lipids. *Am J Primatol* 79:1–15.
- Robeson MS, Khanipov K, Golovko G, Wisely SM, White MD et al., 2018. Assessing the utility of metabarcoding for diet analyses of the omnivorous wild pig *Sus scrofa*. *Ecol Evol* 8:185–196.
- Rogers ME, Maisels F, Williamson EA, Fernandez M, Tutin CEG, 1990. Gorilla diet in the Lopé Reserve, Gabon: a nutritional analysis. *Oecologia* 84:326–339.
- Rothman JM, Chapman CA, Soest PJ, 2012. Methods in primate nutritional ecology: a user's guide. *Int J Primatol* 33:542–566.
- Scanlon AT, Petit S, 2013. How fecal subsampling methods affect the accuracy of dietary pollen detection. *J Mammal* 94:1321–1330.
- Schneider I, Tielen IHM, Rode J, Levelink P, Schrudde D, 2010. Behavioral observations and notes on the vertical ranging pattern of the critically endangered Cat Ba langur *Trachypithecus poliocephalus poliocephalus* in Vietnam. *Primate Conserv* 25:111–117.
- Seefelt N, Gillingham J, 2006. A comparison of three methods to investigate the diet of breeding double-crested cormorants *Phalacrocorax auritus* in the Beaver Archipelago, northern Lake Michigan. *Hydrobiologia* 567:57–67.
- Shannon CE, Weaver W, 1964. *The Mathematical Theory of Communication*. Urbana (IL): The University of Illinois Press.
- Shrestha R, Wegge P, 2006. Determining the composition of herbivore diets in the Trans-Himalayan rangelands: a comparison of field methods. *Rangeland Ecol Manag* 59:512–518.
- Shutt K, Heistermann M, Kasim A, Todd A, Kalousova B et al., 2014. Effects of habituation, research and ecotourism on faecal glucocorticoid metabolites in wild western lowland gorillas: implications for conservation management. *Biol Conserv* 172:72–79.
- Singer MS, Lichter-Marck IH, Farkas TE, Aaron E, Whitney KD et al., 2014. Herbivore diet breadth mediates the cascading effects of carnivores in food webs. *Proc Natl Acad Sci U S A* 111:9521–9526.
- Su H, Lee L, 2001. Food habits of formosan rock macaques *Macaca cyclopis* in Jentse, northeastern Taiwan, assessed by fecal analysis and behavioral observation. *Int J Primatol* 22:359–377.
- Surbeck M, Fowler A, Deimel C, Hohmann G, 2009. Evidence for the consumption of arboreal, diurnal primates by bonobos *Pan paniscus*. *Am J Primatol* 71:171–174.
- Tutin CEG, Fernandez M, 1993. Faecal analysis as a method of describing diets of apes: examples from sympatric gorillas and chimpanzees at Lopé, Gabon. *Tropics* 2:189–197.
- Tuytens FAM, de Graaf S, Heerkens JLT, Jacobs L, Nalon E et al., 2014. Observer bias in animal behaviour research: can we believe what we score, if we score what we believe? *Anim Behav* 90:273–280.
- Tweheyo M, Lye KA, Weladji RB, 2003. Chimpanzee diet and habitat selection in the Budongo Forest Reserve, Uganda. *Forest Ecol Manag* 188:267–278.
- Valentini A, Miquel C, Nawaz M, Bellemain E, Coissac E et al., 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *trmL* approach. *Mol Ecol Resour* 9:51–60.
- van Lieferloo RJ, Schuiling BF, de Boer WF, Lent PC, de Jong CB et al., 2009. A comparison of faecal analysis with backtracking to determine the diet composition and species preference of the black rhinoceros *Diceros bicornis minor*. *Eur J Wildl Res* 55:1–11.
- van Schaik CP, Terborgh JW, Wright SJ, 1993. The phenology of tropical forests: adaptive significance and consequences for primary consumers. *Annu Rev Ecol Evol Syst* 24:353–377.
- VelezLiendo X, Strubbe D, Matthysen E, 2013. Effects of variable selection on modelling habitat and potential distribution of the Andean bear in Bolivia. *Ursus* 24:127–138.
- Wam HK, Hjeljord O, 2010. Moose summer diet from feces and field surveys: a comparative study. *Rangeland Ecol Manag* 63:387–395.
- Ward EJ, Levin PS, Lance MM, Jeffries SJ, Acevedo-Gutiérrez A, 2012. Integrating diet and movement data to identify hot spots of predation risk and areas of conservation concern for endangered species. *Conserv Lett* 5:37–47.

- Watts DP, Potts KB, Lwanga JS, Mitani JC, 2012. Diet of chimpanzees *Pan troglodytes schweinfurthii* at Ngogo, Kibale National Park, Uganda, 1. Diet composition and diversity. *Am J Primatol* 74:114–129.
- Weiser EL, Powell AN, 2011. Evaluating gull diets: a comparison of conventional methods and stable isotope analysis. *J Field Ornithol* 82:297–310.
- Weseloh DV, Ewins PJ, 1994. Characteristics of a rapidly increasing colony of double-crested cormorants *Phalacrocorax auritus* in Lake Ontario: population size, reproductive parameters and band recoveries. *J Great Lakes Res* 20:443–456.
- Wires LR, Cuthbert FJ, Trexel DR, Joshi AR, 2001. Status of the double-crested cormorant (*Phalacrocorax auritus*) in North America. Final Report to USFWS.
- Wrangham RW, Chapman CA, Clark AP, Isabirye-Basuta G, 1996. Social ecology of Kanyawara chimpanzees: implications for understanding the costs of great ape groups. In: McGrew WC, Marchant LF, Nishida T, editors. *Great Ape Societies*. Cambridge (UK): Cambridge University Press, 45–57.
- Wrangham RW, Conklin-Brittain NL, Hunt KD, 1998. Dietary response of chimpanzees and cercopithecines to seasonal variation in fruit abundance. I. Antifeedants. *Int J Primatol* 19:949–970.
- Zuberbühler K, Wittig M, 2011. Field experiments with non-human primates: a tutorial. In: Setchell JM, Curtis DJ, editors. *Field and Laboratory Methods in Primatology: A Practical Guide*. Cambridge (UK): Cambridge University Press.

