

Discrimination factors of carbon and nitrogen stable isotopes in meerkat feces

Shaena Montanari

School of GeoSciences, University of Edinburgh, Edinburgh, United Kingdom

ABSTRACT

Stable isotope analysis of feces can provide a non-invasive method for tracking the dietary habits of nearly any mammalian species. While fecal samples are often collected for macroscopic and genetic study, stable isotope analysis can also be applied to expand the knowledge of species-specific dietary ecology. It is somewhat unclear how digestion changes the isotope ratios of animals' diets, so more controlled diet studies are needed. To date, most diet-to-feces controlled stable isotope experiments have been performed on herbivores, so in this study I analyzed the carbon and nitrogen stable isotope ratios in the diet and feces of the meerkat (*Suricata suricatta*), a small omnivorous mammal. The carbon trophic discrimination factor between diet and feces ($\Delta^{13}\text{C}_{\text{feces}}$) is calculated to be $0.1 \pm 1.5\text{‰}$, which is not significantly different from zero, and in turn, not different than the dietary input. On the other hand, the nitrogen trophic discrimination factor ($\Delta^{15}\text{N}_{\text{feces}}$) is $1.5 \pm 1.1\text{‰}$, which is significantly different from zero, meaning it is different than the average dietary input. Based on data generated in this experiment and a review of the published literature, carbon isotopes of feces characterize diet, while nitrogen isotope ratios of feces are consistently higher than dietary inputs, meaning a discrimination factor needs to be taken into account. The carbon and nitrogen stable isotope values of feces are an excellent snapshot of diet that can be used in concert with other analytical methods to better understand ecology, diets, and habitat use of mammals.

Subjects Animal Behavior, Biogeography, Ecology, Ecosystem Science, Zoology

Keywords Stable isotopes, Ecology, Dietary ecology, Mammalogy, Meerkats, Trophic ecology, Feces

INTRODUCTION

Small mammals are often overlooked in favor of larger more charismatic species, but they fill vital roles as ecosystem engineers, prey base, and seed dispersal agents (*Huntly & Inouye, 1988; Brown & Heske, 1990; Davidson, Detling & Brown, 2012*). They can live in colonies or in large numbers; therefore their plentiful modern and historical remains can provide records of changing environments and shifting ecological conditions (*Terry, 2010*). Non-invasive monitoring is an ideal way to track changes in modern mammalian communities, as shed hair and feces can provide a substrate for examining population trends, diets, and health of groups using genetic or chemical methods (*Crawford, McDonald & Bearhop, 2008; Pompanon et al., 2012; Rodgers & Janečka, 2013*). Specifically examining feces is useful, as it can be collected from rare or cryptic species that are hard to monitor and often avoid humans. Also, feces is plentiful and relatively inexpensive to analyze. Traditionally,

Submitted 22 August 2016

Accepted 17 May 2017

Published 13 June 2017

Corresponding author

Shaena Montanari,

shae.montanari@gmail.com

Academic editor

Rita Grandori

Additional Information and
Declarations can be found on
page 11

DOI 10.7717/peerj.3436

© Copyright

2017 Montanari

Distributed under

Creative Commons CC-BY 4.0

OPEN ACCESS

vertebrate diets have been assessed macroscopically through physical examination of gut contents and feces (e.g., [Hermsen, Kerle & Old, 2016](#)). With the advent of affordable high-throughput sequencing, fecal studies are becoming more common for barcoding dietary DNA, which allows for a more complete dietary picture not as easily biased by differing digestibility of food (e.g., [Shehzad et al., 2012](#); [Kartzinel et al., 2015](#)). Stable isotope methods can provide a useful complement to these barcoding studies that are growing in popularity. Fecal stable isotopes have been useful for detecting dietary shifts in mammals such as gorillas ([Blumenthal et al., 2012](#)), for understanding weaning time in primates ([Reitsema, 2012](#)), and as a climate record from bats ([Royer et al., 2015](#)), so understanding isotopic discrimination and variability of this material is vital for future research.

Stable isotope ratios of nitrogen ($^{15}\text{N}/^{14}\text{N}$, written $\delta^{15}\text{N}$) and carbon ($^{13}\text{C}/^{12}\text{C}$, written $\delta^{13}\text{C}$) are incorporated in animal tissues and excretions following digestion of food products. Changes in stable isotope ratios can elucidate information about food webs, trophic structure, and habitat use ([Ben-David & Flaherty, 2012](#)). It is assumed that carbon isotope ratios do not change drastically as they propagate through the food web ([DeNiro & Epstein, 1978](#)), and in terrestrial ecosystems, these ratios are generally used to indicate the primary production at the base of the food web. Carbon isotope ratios of plants that use different metabolic pathways (C3, C4, or CAM) are systematically different, and this difference is incorporated into the tissues or byproducts of consumers. On the other hand, $\delta^{15}\text{N}$ values are known to become enriched as trophic level increases ([DeNiro & Epstein, 1981](#)), and differences in $\delta^{15}\text{N}$ of tissue over time or between populations can be used to discern changes in food webs, habitat, or prey composition ([Post, 2002](#)).

To use stable isotopes in dietary ecology, there has to be a comprehensive understanding of the difference in isotope ratios between diet and tissue (or feces); this difference is called the trophic discrimination factor (also fractionation factor or discrimination factor) and is caused by isotopic fractionation during digestion and metabolism. Trophic discrimination factor is denoted as Δ and defined as $\Delta = \delta_{\text{tissue}} - \delta_{\text{diet}}$ ([Martínez del Rio et al., 2009](#)). Trophic discrimination factors (abbreviated TDFs) are widely variable among animals and differ depending on species, tissue examined, and diet type and quality ([Caut, Angulo & Courchamp, 2009](#)). General TDFs are used in many mixing model studies for animal diet reconstruction, but it has been shown that small differences in TDFs in these types of studies can lead to vastly different conclusions about dietary makeup ([Ben-David & Schell, 2001](#)). Experimental work by [Caut, Angulo & Courchamp \(2008\)](#) shows that mixing models are most accurate when species-specific TDFs are obtained.

In this study, I calculate TDFs for carbon and nitrogen isotopes in meerkat (*Suricata suricatta*) feces in order to obtain fecal TDFs in a small mammalian omnivore. Meerkats are small, diurnal mammals that are members of Carnivora and are herpestids, closely related to mongoose. They have generalist diets and in the wild consume insects, berries, reptiles, and other small invertebrates ([Doolan & Macdonald, 1996](#)). The diets of generalist consumers are difficult to study in the wild due to unknown variability in their diet, so a zoo-based study of meerkats in this instance will provide dietary control, allowing for less variation in the determination of a TDF. Studies like this one are necessary, as TDFs are not often determined for terrestrial mammalian omnivores and carnivores or even feces in

Table 1 Fecal trophic discrimination factors of carbon ($\Delta^{13}\text{C}$) from this study and from the literature. Average $\delta^{13}\text{C}$ of diet used to calculate each TDF is included from the original listed publication. All isotope values are presented ± 1 standard deviation if it was available in the original publication. Asterisks (*) indicate when one species from the same experiment was divided into different diet treatments. The column “n” represents the number of fecal samples used to calculate the TDF in each experiment. The $\Delta^{13}\text{C}$ values in bold are statistically significantly different from zero in their original experiments (in some cases this was not specifically tested).

Species	$\delta^{13}\text{C}$ diet	$\Delta^{13}\text{C}$	n	Diet class	Reference
<i>Peromyscus maniculatus</i>	-19.4 ± 0.3	-3.2	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Myodes gapperi</i>	-19.4 ± 0.3	-4.2	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Microtus longicaudus</i>	-19.4 ± 0.3	-2.7	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Microtus pennsylvanicus</i>	-19.4 ± 0.3	-3.6	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Tamias amoenus</i>	-19.4 ± 0.3	-3.0	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Zapus princeps</i>	-19.4 ± 0.3	-5.9	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Lama glama</i> *	-13.3 ± 0.3	-1.2 ± 0.4	4	Herbivore	Sponheimer et al. (2003a)
<i>Lama glama</i> *	-27.0 ± 0.2	-0.4 ± 0.5	4	Herbivore	Sponheimer et al. (2003a)
<i>Capra hircus</i> *	-27.0 ± 0.2	-0.8 ± 0.1	4	Herbivore	Sponheimer et al. (2003a)
<i>Capra hircus</i> *	-13.3 ± 0.3	-1.0 ± 0.4	4	Herbivore	Sponheimer et al. (2003a)
<i>Bos taurus</i> *	-13.3 ± 0.3	-0.9 ± 0.2	4	Herbivore	Sponheimer et al. (2003a)
<i>Bos taurus</i> *	-27.0 ± 0.2	-1.00 ± 0.2	4	Herbivore	Sponheimer et al. (2003a)
<i>Oryctolagus cuniculus</i>	-27.0 ± 0.2	-0.3 ± 0.1	4	Herbivore	Sponheimer et al. (2003a)
<i>Vicugna pacos</i> *	-27.0 ± 0.2	-0.4 ± 0.4	4	Herbivore	Sponheimer et al. (2003a)
<i>Vicugna pacos</i> *	-13.3 ± 0.3	-1.3 ± 0.2	4	Herbivore	Sponheimer et al. (2003a)
<i>Equus caballus</i> *	-27.0 ± 0.2	-0.5 ± 0.4	4	Herbivore	Sponheimer et al. (2003a)
<i>Equus caballus</i> *	-13.3 ± 0.3	-0.7 ± 0.2	4	Herbivore	Sponheimer et al. (2003a)
<i>Uncia uncia</i>	-23.61 ± 3.15	2.30 ± 1.66	10	Carnivore	Montanari & Amato (2015)
<i>Panthera tigris</i>	-23.61 ± 3.15	1.25 ± 0.62	7	Carnivore	Montanari & Amato (2015)
<i>Gorilla gorilla</i>	-28.4	0.3	121	Herbivore	Blumenthal et al. (2012)
<i>Clethrionomys gapperi</i> *	-29.23	-0.51 ± 1.19	11	Herbivore	Sare, Millar & Longstaffe (2005)
<i>Clethrionomys gapperi</i> *	-25.49	-1.95 ± 1.04	10	Herbivore	Sare, Millar & Longstaffe (2005)
<i>Clethrionomys gapperi</i> *	-28.22	0.24 ± 1.20	10	Herbivore	Sare, Millar & Longstaffe (2005)
<i>Myotis myotis</i> *	-24.54 ± 0.76	-0.17 ± 1.10	15	Insectivore	Salvarina et al. (2013)
<i>Myotis myotis</i> *	-20.50 ± 0.81	-0.25 ± 0.75	21	Insectivore	Salvarina et al. (2013)
<i>Rhinolophus ferrumequinum</i> *	-24.54 ± 0.76	0	15	Insectivore	Salvarina et al. (2013)
<i>Rhinolophus ferrumequinum</i> *	-20.50 ± 0.81	0.09 ± 0.39	21	Insectivore	Salvarina et al. (2013)
<i>Suricata suricatta</i>	-24.9 ± 3.3	0.1 ± 1.5	24	Omnivore	This study

general (Tables 1 and 2). Even though feces represent a snapshot of diet, it is often collected in sizeable quantities for non-invasive studies. This can provide important ecological and environmental records and elucidate short-term and seasonal dietary variability. Changes in isotope ratios from diet to feces need to be calculated, as there may be isotopic fractionation from the process of digestion and waste excretion.

Here I calculate trophic discrimination factors for captive meerkats by measuring the isotopic composition of both the diet and the feces. I also describe how fecal stable isotope values vary over short periods of time so that more information can be gleaned about the measured variability in generalist diets during wild studies. Focusing on small mammals with generalist diets will be key to uncovering ecological factors that cause shifts

Table 2 Fecal trophic discrimination factors of nitrogen ($\Delta^{15}\text{N}$) from this study and from the literature. Average $\delta^{15}\text{N}$ of diet used to calculate each TDF is included from the original listed publication. All isotope values are presented 1 standard deviation if it was available in the original publication. Asterisks (*) indicate when one species from the same experiment was divided into different diet treatments. The column “n” represents the number of fecal samples used to calculate the TDF in each experiment. The $\Delta^{15}\text{N}$ values in bold are statistically significantly different from zero in their original experiments (in some cases this was not specifically tested).

Species	$\delta^{15}\text{N}$ diet	$\Delta^{15}\text{N}$	n	Diet class	References
<i>Peromyscus maniculatus</i>	3.6 ± 0.02	2.1	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Myodes gapperi</i>	3.6 ± 0.02	2.2	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Microtus longicaudus</i>	3.6 ± 0.02	2.2	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Microtus pennsylvanicus</i>	3.6 ± 0.02	2.5	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Tamias amoenus</i>	3.6 ± 0.02	1.4	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Zapus princeps</i>	3.6 ± 0.02	2.2	5	Herbivore	Hwang, Millar & Longstaffe (2007)
<i>Lama glama</i> *	0.4	2.9 ± 0.3	4	Herbivore	Sponheimer et al. (2003b)
<i>Lama glama</i> *	5.8	3.0 ± 0.4	4	Herbivore	Sponheimer et al. (2003b)
<i>Bos taurus</i> *	0.7	2.0	4	Herbivore	Steele & Daniel (1978)
<i>Bos taurus</i> *	0.6	1.7	4	Herbivore	Steele & Daniel (1978)
<i>Equus caballus</i> *	0.4	2.6	Unknown	Herbivore	Sponheimer et al. (2003b)
<i>Equus caballus</i> *	5.8	3.3	Unknown	Herbivore	Sponheimer et al. (2003b)
<i>Ovis aries</i>	0.8	3.0	Unknown	Herbivore	Sutoh, Obara & Yoneyama (1993)
<i>Capra hircus</i>	1.5	3.6	3	Herbivore	Sutoh, Koyama & Yoneyama (1987)
<i>Uncia uncia</i>	8.95 ± 0.73	2.49 ± 1.30	10	Carnivore	Montanari & Amato (2015)
<i>Panthera tigris</i>	8.95 ± 0.73	1.57 ± 2.04	7	Carnivore	Montanari & Amato (2015)
<i>Sus scrofa</i>	4.6 ± 0.3	1.2	3	Omnivore	Sutoh, Koyama & Yoneyama (1987)
<i>Gorilla gorilla</i>	3.2	0.6	121	Herbivore	Blumenthal et al. (2012)
<i>Clethrionomys gapperi</i> *	-0.42	1.76 ± 1.26	11	Herbivore	Sare, Millar & Longstaffe (2005)
<i>Clethrionomys gapperi</i> *	1.45	1.17 ± 1.68	10	Herbivore	Sare, Millar & Longstaffe (2005)
<i>Clethrionomys gapperi</i> *	4.00	1.27 ± 2.06	10	Herbivore	Sare, Millar & Longstaffe (2005)
<i>Myotis myotis</i> *	5.31 ± 0.63	1.81 ± 1.28	15	Insectivore	Salvarina et al. (2013)
<i>Myotis myotis</i> *	12.88 ± 1.16	2.34 ± 2.17	21	Insectivore	Salvarina et al. (2013)
<i>Rhinolophus ferrumequinum</i> *	5.31 ± 0.63	0.53 ± 0.54	14	Insectivore	Salvarina et al. (2013)
<i>Rhinolophus ferrumequinum</i> *	12.88 ± 1.16	0.97 ± 0.45	21	Insectivore	Salvarina et al. (2013)
<i>Suricata suricatta</i>	4.6 ± 1.8	1.5 ± 1.1	24	Omnivore	This study

in mammalian biodiversity, as small mammals are accurate recorders of environmental change over a variety of time scales (Barnosky, Hadly & Bell, 2003; Terry, 2010).

MATERIALS AND METHODS

Diet and feces samples

The meerkats in this study are maintained at the Edinburgh Zoo (Royal Zoological Society Scotland). Fecal samples were taken randomly from an enclosure containing seven adult female meerkats over the course of April 2016. Subsamples of diet were collected once a week for four weeks to account for variability in diet items. Per the information of Edinburgh Zoo animal care workers, the meerkats are fed different combinations of the food items each day, but over the course of a week the amount of each item they eat is roughly equal. Each animal receives the same amount of each food item by weight. A total

of 24 fecal samples were collected, along with 2–4 subsamples of each diet item: carrots and apples, horsemeat, dog biscuits, whole frozen small mice, and whole frozen chicks. The above items were used to calculate the trophic discrimination factor from diet to feces. Homogenized bulk muscle with attached skin was sectioned from the whole chicks and mice for analysis.

Stable isotope analysis

Stable isotope analysis was conducted at the Wolfson Laboratory in the School of Geosciences at the University of Edinburgh. The analysis of carbon and nitrogen isotope ratios was performed on a CE Instruments NA2500 Elemental Analyzer and the effluent gas was analyzed for its carbon and nitrogen isotopic ratios using a Thermo Electron Delta+ Advantage stable isotope ratio mass spectrometer. Sediment standard, PACS-2 ($\delta^{15}\text{N}$ 5.215‰ (Air) and $\delta^{13}\text{C}$ - 22.228‰ (VPDB)) from the National Research Council Canada was used for isotopic analyses. Acetanilide standard (C 71.09% and N 10.36%) was used for elemental compositions. Isotopic data were determined relative to CO_2 and N_2 reference gases whose mean values are derived from the average value of PACS-2 samples within each daily run.

The standard deviation for five analyses of the PACS-2 standard run over the same time period as the study samples was $\pm 0.07\text{‰}$ for $\delta^{13}\text{C}$ (VPDB), and $\pm 0.14\text{‰}$ for $\delta^{15}\text{N}$ (Air). Elemental analysis, measuring the percentage of carbon and nitrogen in the samples, had an error of 1% for carbon and 4% for nitrogen. The stable isotope ratios are reported in standard notation and referenced to air for $\delta^{15}\text{N}$ values and Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$ values. Ratios are defined as $\delta = (R_{\text{sample}}/R_{\text{standard}} - 1)$ where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$.

Samples were prepared first by lyophilization followed by manual crushing to form a homogenized powder for isotope analysis. Feces and diet samples were subsampled and analyzed both with and without lipid extraction treatment. The samples were lipid extracted by immersion in a 2:1 ratio of chloroform/methanol for 12 h using a Soxhlet apparatus. Following lipid extraction, samples were dried in a 50 °C oven for at least 24 h to evaporate any remaining solvent.

Statistics and data analysis

Statistical tests and analyses were performed in R (*R Core Team, 2016*), and plots were created in ggplot2 (*Wickham, 2009*). Trophic discrimination factors were calculated using the averages of all subsampled dietary material. TDFs were calculated for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using the aforementioned equation $\Delta X_{\text{feces}} = \delta X_{\text{feces}} - \delta X_{\text{diet}}$ where ΔX_{feces} is calculated in per mil (‰). δX_{diet} is the value of the average of all non-lipid-extracted dietary samples and δX_{feces} is each individual fecal stable isotope value.

Parametric statistical tests were performed, as the data are normally distributed as shown through a Shapiro–Wilk test ($\delta^{13}\text{C}$: $W = 0.946$, $p = 0.057$; $\delta^{15}\text{N}$: $W = 0.976$, $p = 0.544$). Before hypothesis testing, an F -test was performed to see if a t -test for equal or unequal variance (Welch two sample t -test) should be done based on the results. F -tests show the variance was equal for comparisons between lipid-extracted and non-lipid-extracted feces

for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ($\delta^{13}\text{C}$: $F_{8,23} = 1.409$, $p = 0.491$; $\delta^{15}\text{N}$: $F_{8,23} = 0.408$, $p = 0.191$). Variance for comparisons between diet and feces for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are unequal ($\delta^{13}\text{C}$: $F_{15,23} = 5.001$, $p = 0.0006$; $\delta^{15}\text{N}$: $F_{15,23} = 2.633$, $p = 0.036$). All means are reported \pm standard deviation (SD), and all significance is reported for $\alpha = 0.05$.

To obtain carbon and nitrogen fecal trophic discrimination factors from other studies for comparison to results from this study, a literature search was conducted using Google Scholar and through mining references in other feces trophic discrimination factor papers. These were all of the papers with diet-feces trophic discrimination factors found as of August 2016 searching keywords such as “feces stable isotopes” and “scat stable isotopes”. These values appear in [Tables 1 and 2](#).

RESULTS

A summary of the stable isotope results is presented in [Table 3](#), and a summary of t -tests is reported in [Table 4](#). All raw isotope data can be found in the supplementary information ([Data S1](#)).

A subset of feces and diet samples were lipid extracted to see if this changed the results of the analysis; however, using a t -test no significant difference was found between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of either material between lipid extracted and non-lipid-extracted samples ([Table 4](#)). As in my previous study of fecal TDFs ([Montanari & Amato, 2015](#)), I have opted to use only non-lipid-extracted materials for sampling, as a meta-analysis of carbon and nitrogen discrimination factors from [Caut, Angulo & Courchamp \(2009\)](#) has shown that lipid extraction has no significant effect on calculated TDFs.

The mean $\delta^{13}\text{C}$ value for each food item is as follows: chick ($n = 2$, $-26.1 \pm 1.2\text{‰}$), mouse ($n = 2$, $-23.4 \pm 1.7\text{‰}$), horsemeat ($n = 4$, $-27.1 \pm 1.3\text{‰}$), carrots and apple mix ($n = 4$, $-27.7 \pm 1.5\text{‰}$), and dog biscuits ($n = 4$, $-20.2 \pm 0.5\text{‰}$). The average for all diet items ($n = 16$) is $-24.9 \pm 3.3\text{‰}$. Mean $\delta^{15}\text{N}$ values are: chick ($4.5 \pm 0.03\text{‰}$), mouse ($5.2 \pm 0.6\text{‰}$), horsemeat ($7.0 \pm 1.3\text{‰}$), carrots and apple mix ($3.5 \pm 1.0\text{‰}$), and dog biscuits ($2.9 \pm 0.5\text{‰}$). The average $\delta^{15}\text{N}$ for all diet items is $4.6 \pm 1.8\text{‰}$. The mean diet C/N ratio is 27.1 ± 38.1 . The mean $\delta^{13}\text{C}$ value of the feces is $-24.8 \pm 1.5\text{‰}$ and the mean $\delta^{15}\text{N}$ value is $6.1 \pm 1.1\text{‰}$. The C/N ratio is 6.9 ± 1.6 . The ranges and variability of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for all materials are presented in [Table 3](#) and the variation of fecal isotope values over the month they were collected is presented in [Fig. 1](#). When the fecal samples were placed into bins by weeks they were collected and subjected to an ANOVA, there was no difference between the average weekly scat values over the course of the month ($\delta^{13}\text{C}$: $F_{4,19} = 0.539$, $p = 0.709$; $\delta^{15}\text{N}$: $F_{4,19} = 0.886$, $p = 0.491$).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of feces and diet were compared to establish if there is a significant difference between them. In the case of $\delta^{13}\text{C}$ there is no significant difference (Welch two-sample t -test, $t = -0.14$, $df = 19.05$, $p = 0.89$) while for $\delta^{15}\text{N}$ the means are significantly different (Welch two sample t -test, $t = -2.97$, $df = 22.61$, $p = 0.01$). Discrimination factors were calculated by using the average value of all diet items subtracted from each individual feces sample in order to assess variance. Average $\Delta^{13}\text{C}$ for feces ($\Delta^{13}\text{C}_{\text{feces}}$) is $0.1 \pm 1.5\text{‰}$ and $\Delta^{15}\text{N}$ for feces ($\Delta^{15}\text{N}_{\text{feces}}$) is $1.5 \pm 1.1\text{‰}$, with only the $\Delta^{15}\text{N}_{\text{feces}}$ being significantly different than zero.

Table 3 Stable isotope results from meerkat feces and diet samples ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Means are shown ± 1 standard deviation. Stable isotopes are presented in delta notation (δ) and discrimination factors are noted by Δ . All isotope values are presented in per mil (‰). Trophic discrimination factor in bold is statistically significant from zero.

	<i>n</i>	$\delta^{13}\text{C}$ (‰)	$\delta^{13}\text{C}$ range	$\delta^{15}\text{N}$ (‰)	$\delta^{15}\text{N}$ range	C/N	C%	N%	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$
Meerkat scat	24	-24.8 ± 1.5	-28.1,-20.7	6.1 ± 1.1	4.4,8.9	6.9 ± 1.6	20.9 ± 11.1	3.2 ± 1.8	0.1 ± 1.5	1.5 ± 1.1
Chick	2	-26.1 ± 1.2	-27.0,-25.3	4.5 ± 0.03	4.5	3.7 ± 0.3	47.8 ± 0.3	12.9 ± 1.0		
Mouse	2	-23.4 ± 1.7	-24.6,-22.2	5.2 ± 0.6	4.7,5.6	6.1 ± 1.5	51.4 ± 4.7	8.7 ± 1.3		
Horse meat	4	-27.1 ± 1.3	-28.2,-25.3	7.0 ± 1.3	6.1,9.0	3.5 ± 0.04	46.0 ± 1.9	13.3 ± 0.4		
Fruit mix	4	-27.7 ± 1.5	-29.8,-26.8	3.5 ± 1.0	2.8,4.9	88.6 ± 21.8	38.5 ± 1.0	0.5 ± 0.1		
Dog biscuits	4	-20.2 ± 0.5	-20.7,-19.5	2.9 ± 0.5	2.3,3.4	11.4 ± 1.8	42.9 ± 0.7	3.8 ± 0.6		
Total diet	16	-24.9 ± 3.3	-29.8,-19.5	4.6 ± 1.8	2.3,9.0	27.1 ± 38.1	44.2 ± 4.6	7.1 ± 5.5		

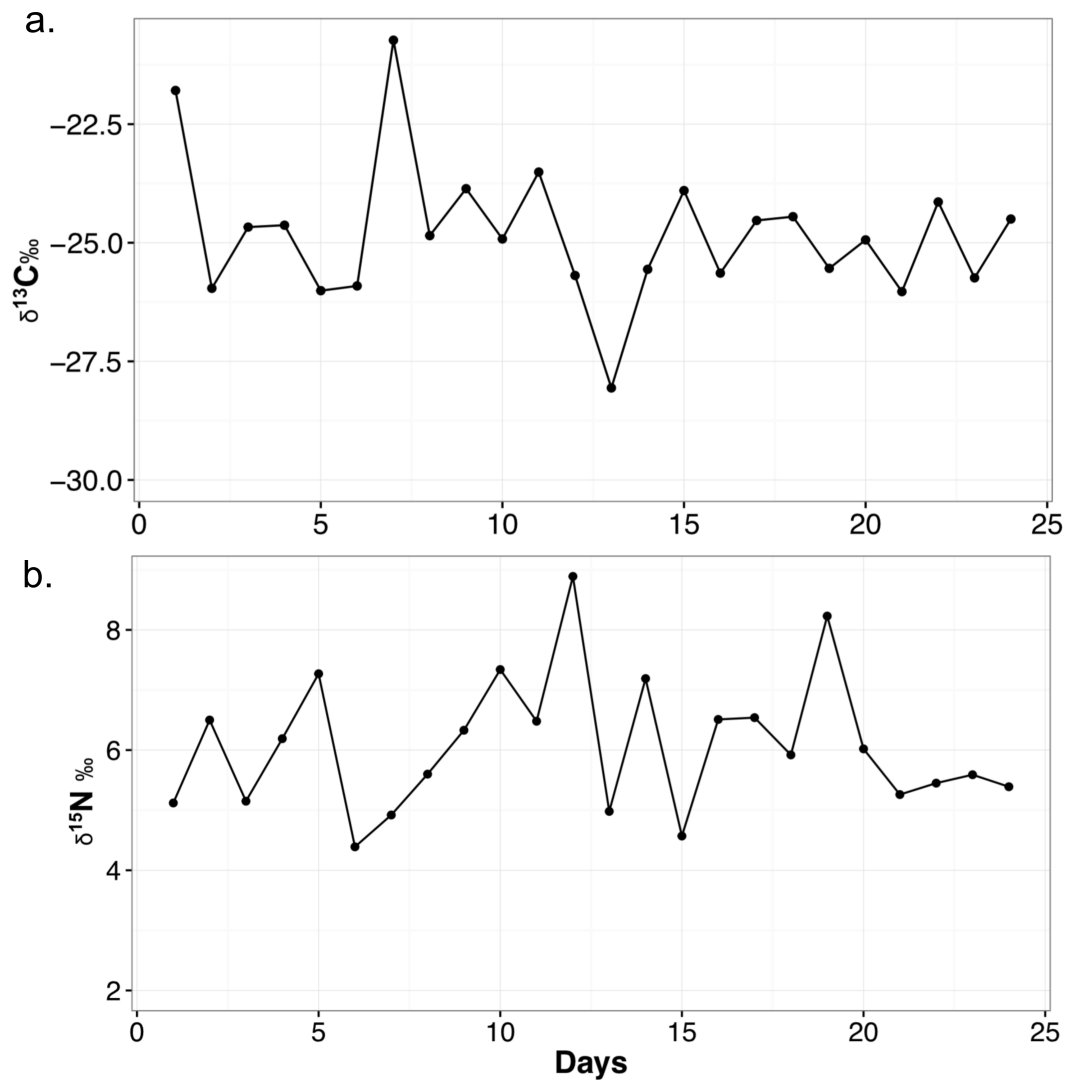


Figure 1 Variability in $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values of meerkat feces over the month of sampling. Points on the line represented a measured fecal sample. All isotope values are presented in per mil (‰).

Table 4 Results from t -tests (p -value, t , df) comparing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ means of stable isotope values from lipid and non-lipid-extracted meerkat feces and diet samples. The t -test used (Welch or equal) was decided by a preliminary F -test to test for equal variances.

Variable 1	Variable 2	Test	p -value	t	df
$\delta^{13}\text{C}$ Scat LE	$\delta^{13}\text{C}$ Scat	t -test equal	0.12	1.59	31
$\delta^{15}\text{N}$ Scat LE	$\delta^{15}\text{N}$ Scat	t -test equal	0.09	-1.74	31
$\delta^{13}\text{C}$ Diet LE	$\delta^{13}\text{C}$ Diet	t -test equal	0.46	0.75	14
$\delta^{15}\text{N}$ Diet LE	$\delta^{15}\text{N}$ Diet	t -test equal	0.79	0.27	14
$\delta^{13}\text{C}$ Diet	$\delta^{13}\text{C}$ Scat	Welch t -test	0.89	-0.14	19.05
$\delta^{15}\text{N}$ Diet	$\delta^{15}\text{N}$ Scat	Welch t -test	0.01	-2.97	22.61

DISCUSSION

Trophic discrimination factors of meerkat feces

Feces can be an extremely useful substrate for stable isotope analysis for estimating food input, and these data show in meerkats that in relation to carbon isotopes, feces are a fair representation of diet, but nitrogen isotopes of feces undergo isotopic enrichment. Investigations into stable isotope discrimination factors show that herbivore feces are representative of diet in large bodied animals (*Sponheimer et al., 2003a*) but that in small-bodied herbivores (*Hwang, Millar & Longstaffe, 2007*) and non-herbivores (*Montanari & Amato, 2015*) feces undergo enrichment of nitrogen isotopes within the digestive tract. In this study, the trophic discrimination factor of carbon isotopes from diet-to-feces is small and not statistically significant. Similar results for $\Delta^{13}\text{C}_{\text{feces}}$ are seen in studies of insectivores (bats, *Salvarina et al., 2013*) and carnivores (tigers and snow leopards, *Montanari & Amato, 2015*). To this point, experimental research on diet-to-feces discrimination factors shows most calculated carbon TDFs are non-significant as referenced by the review of published studies in [Table 1](#). More data are needed on mammalian omnivores and carnivores to further assess this pattern.

The means of $\delta^{15}\text{N}_{\text{diet}}$ and $\delta^{15}\text{N}_{\text{feces}}$ are different ([Table 4](#)); therefore the $\Delta^{15}\text{N}_{\text{feces}}$ value ($1.5 \pm 1.1\text{‰}$) is significantly different than zero. In this study and the other two non-herbivore fecal TDF studies of *Salvarina et al. (2013)* and *Montanari & Amato (2015)*, $\Delta^{15}\text{N}_{\text{feces}}$ is of similar magnitude and also the only TDF that is different than zero; although $\Delta^{15}\text{N}_{\text{feces}}$ is only significant in one out of two species examined in each of these studies. Nevertheless, significance of $\Delta^{15}\text{N}_{\text{feces}}$ could indicate ^{15}N enrichment occurs during digestion, likely during transit through the gut as is seen in *Hwang, Millar & Longstaffe (2007)*. This study investigated $\delta^{15}\text{N}$ values at different parts in the digestive tract of voles and other small rodents and found digested material is enriched in ^{15}N in the stomach, intestine, cecum, and colon relative to the diet. It has also been shown the $\delta^{15}\text{N}$ of mucosal epithelium was higher than the diet input in some parts of the digestive tract of a sheep, which suggests the enrichment in ^{15}N in feces may be due to the presence of endogenous proteins (*Sutoh, Obara & Yoneyama, 1993*). In general, significant $\Delta^{15}\text{N}$ values point to some relationship between digestive processes and $\Delta^{15}\text{N}$, but the cause is unknown and more experiments comparing ^{15}N enrichment during digestion in herbivores and non-herbivores are needed.

A number of other factors may be also affecting nitrogen flux during digestion and excretion. Different biochemical pathways related to the changes in proteins occurring during digestion (deamination/amination) could be the cause of ^{15}N enrichment (*Hwang, Millar & Longstaffe, 2007*). Additionally, the presence of microorganisms in the digestive tract could also enrich fecal matter compared to diet (*Hwang, Millar & Longstaffe, 2007*; *Macko et al., 1987*). The ^{15}N enrichment could be due to preferential absorption of ^{14}N by the animal during digestion or removal during urine excretion, but experimental data from *Sponheimer et al. (2003b)* seem to indicate that at least in herbivores, ^{14}N is not preferentially excreted. In *Montanari & Amato (2015)*, we cautioned that more data should be collected to better understand TDFs of carnivores because the physiological mechanisms

are still unknown; this study continues in the same vein and reinforces that there is a preferential loss of ^{14}N and/or enrichment in ^{15}N occurring during movement through the gastrointestinal tract in relation to solid waste. Further mammalian physiological experiments may explain the mechanism.

Variables that affect trophic discrimination factors

It has been shown that TDFs vary due to a number of factors, one of them being the initial isotopic ratio of the diet. Significant relationships between both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of diets and TDFs have been shown in bears (*Hilderbrand et al., 1996; Felicetti et al., 2003*), rats (*Caut, Angulo & Courchamp, 2008*), and birds (*Pearson et al., 2003*). A meta-analysis of TDFs and dietary isotope ratios values by *Caut, Angulo & Courchamp (2009)* finds significant negative relationships between these variables in both carbon and nitrogen. A similar study of this relationship with fecal TDFs cannot be completed due to the fact most of the calculated fecal TDFs are not significantly different from 0 (data from [Tables 1](#) and [2](#)). This could be due to the fact that feces represents immediately ingested diet (within hours or days) as opposed to assimilated diet and is not subjected to as many physiological processes of fractionation. This is a promising result for the use of feces in isotope studies because the variability of TDFs due to dietary input is a major issue for stable isotope food web studies. Variability that needs to be accounted for with other tissues (*Caut, Angulo & Courchamp, 2009*) appears to be a non-issue in feces.

Mammalian body size might influence calculated TDF (*Hwang, Millar & Longstaffe, 2007*). A mechanism that could explain this is a general trend of higher mass-specific metabolism in animals with smaller body mass (Kleiber's law), which could in turn impact the fractionation of isotopes that occurs after food ingestion (*Pecquerie et al., 2010*). *Hwang, Millar & Longstaffe (2007)* did a meta-analysis of fecal TDFs in the literature combined with their rodent TDFs and found significant differences in $\Delta^{13}\text{C}$ between different body sizes, but only in herbivores. A lack of published fecal TDFs for non-herbivorous mammals of different sizes means statistical test cannot be performed for herbivores vs. non-herbivores.

Isotopic variability over time

Feces can be used for point estimates of diet, but long term collection is especially useful for finding seasonal patterns in dietary variability (e.g., *Blumenthal et al., 2012*). Mammalian carnivores and omnivores tend to change their diets seasonally, so it is important to realize the magnitude of fecal isotope variation day-to-day for wild studies (e.g., *Kincaid & Cameron, 1982; Melero et al., 2008*). Other than this study, *Blumenthal et al. (2012)* and *Salvarina et al. (2013)* track stable isotope ratios of animal feces at monthly or daily intervals respectively.

I have tracked meerkat fecal stable isotopes over the course of one month, and it is clear there is variation on any given day a sample is taken ([Fig. 1](#)), as the combination of diet items they eat changes daily. There is a range of 7.3‰ in the $\delta^{13}\text{C}$ and 4.5‰ $\delta^{15}\text{N}_{\text{feces}}$ of meerkat feces over a month. This suggests feces can be directly reflective of a highly variable diet, and also shows $\delta^{13}\text{C}_{\text{feces}}$ may not be buffered in small mammals as much against day-to-day variability as in larger mammals (*Blumenthal et al., 2012*). It is not

known exactly how long it takes isotopes of feces to reach dietary equilibrium in meerkats, but in *Salvarina et al. (2013)* it was shown that bat feces acquired a new dietary signal in 2–3 h so it stands to reason it is also within hours for a small mammal. Examining the daily fluctuations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in feces indicates timing of major dietary changes can be pinpointed quite precisely using this method, at least within days. Due to the fact the TDFs in this study were calculated using an average diet value, the TDF is also averaged and is meant to act as a general guide for a TDF in the wild. This variability is important to realize for wild studies, as it emphasizes the need for larger sample sizes of feces, such as multiple samples per day or week, to lessen the impact of day-to-day variability if researchers are seeking a long-term ecological or environmental trend (e.g., *Blumenthal et al., 2012*).

CONCLUSIONS

I found the $\delta^{13}\text{C}_{\text{feces}}$ of captive meerkats was not changed compared to the dietary input, while $\delta^{15}\text{N}_{\text{feces}}$ is higher than diet. Compared with other published fecal TDFs, the meerkat data fit with observed trophic discrimination factors, and also show that an enrichment of ^{15}N and/or a depletion of ^{14}N is occurring during digestion or in the gut during gastrointestinal transit. When these TDFs are compared to other fecal TDFs in published literature, they are similar in that they are mostly non-significant, which removes one layer of uncertainty when using feces in wild animal studies. Looking at the stable isotope ratios for both carbon and nitrogen over the course of the month, it is clear short-term, near-daily variability in diet can be captured using stable isotope analysis of meerkat feces. Captive studies like this one with more controlled feces and diet collection parameters will hopefully lead to better understanding in other understudied groups, like terrestrial small and medium sized mammals with omnivorous diets, so that stable isotope analysis of feces can become a more common tool in mammalian stable isotope ecology.

ACKNOWLEDGEMENTS

The author thanks the animal care staff at Edinburgh Zoo for collecting the diet and feces samples and facilitating this research project. Thanks are extended to Colin Chilcott and Steve Mowbray at the University of Edinburgh for help preparing and analyzing all samples stable isotope analysis. The author gives many thanks for reviews and comments from Roberto Ambrosini and two other anonymous reviewers that greatly improved this manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

Funding for this work was provided by the Royal Society Newton International Fellowship. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the author:
Royal Society Newton International Fellowship.

Competing Interests

The author declares that she has no competing interests.

Author Contributions

- Shaena Montanari conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:
The raw data has been supplied as [Data S1](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.3436#supplemental-information>.

REFERENCES

- Barnosky AD, Hadly EA, Bell CJ. 2003.** Mammalian response to global warming on varied temporal scales. *Journal of Mammalogy* **84**:354–368
[DOI 10.1644/1545-1542\(2003\)084<0354:MRTGWO>2.0.CO;2](https://doi.org/10.1644/1545-1542(2003)084<0354:MRTGWO>2.0.CO;2).
- Ben-David M, Flaherty EA. 2012.** Stable isotopes in mammalian research: a beginner's guide. *Journal of Mammalogy* **93**:312–328 [DOI 10.1644/11-MAMM-S-166.1](https://doi.org/10.1644/11-MAMM-S-166.1).
- Ben-David M, Schell DM. 2001.** Mixing models in analyses of diet using multiple stable isotopes: a response. *Oecologia* **127**:180–184 [DOI 10.1007/s004420000570](https://doi.org/10.1007/s004420000570).
- Blumenthal SA, Chritz KL, Rothman JM, Cerling TE. 2012.** Detecting intraannual dietary variability in wild mountain gorillas by stable isotope analysis of feces. *Proceedings of the National Academy of Sciences of the United States of America* **109**:21277–21282 [DOI 10.1073/pnas.12,15782109](https://doi.org/10.1073/pnas.12,15782109).
- Brown JH, Heske EJ. 1990.** Control of a desert-grassland transition by a keystone rodent guild. *Science* **250**:1705–1707 [DOI 10.1126/science.250.4988.1705](https://doi.org/10.1126/science.250.4988.1705).
- Caut S, Angulo E, Courchamp F. 2008.** Discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$) in an omnivorous consumer: effect of diet isotopic ratio. *Functional Ecology* **22**:255–263 [DOI 10.1111/j.1365-2435.2007.01360.x](https://doi.org/10.1111/j.1365-2435.2007.01360.x).
- Caut S, Angulo E, Courchamp F. 2009.** Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology* **46**:443–453 [DOI 10.1111/j.1365-2664.2009.01620.x](https://doi.org/10.1111/j.1365-2664.2009.01620.x).
- Crawford K, McDonald RA, Bearhop S. 2008.** Applications of stable isotope techniques to the ecology of mammals. *Mammal Review* **38**:87–107
[DOI 10.1111/j.1365-2907.2008.00120.x](https://doi.org/10.1111/j.1365-2907.2008.00120.x).

- Davidson AD, Detling JK, Brown JH. 2012.** Ecological roles and conservation challenges of social, burrowing, herbivorous mammals in the world's grasslands. *Frontiers in Ecology and the Environment* **10**:477–486 DOI [10.1890/110054](https://doi.org/10.1890/110054).
- DeNiro MJ, Epstein S. 1978.** Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* **42**:495–506 DOI [10.1016/0016-7037\(78\)90199-0](https://doi.org/10.1016/0016-7037(78)90199-0).
- DeNiro MJ, Epstein S. 1981.** Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica Et Cosmochimica Acta* **45**:341–351 DOI [10.1016/0016-7037\(81\)90244-1](https://doi.org/10.1016/0016-7037(81)90244-1).
- Doolan SP, Macdonald DW. 1996.** Diet and foraging behaviour of group-living meerkats, *Suricata suricatta*, in the southern Kalahari. *Journal of Zoology* **239**:697–716 DOI [10.1111/j.1469-7998.1996.tb05472.x](https://doi.org/10.1111/j.1469-7998.1996.tb05472.x).
- Felicetti LA, Schwartz CC, Rye RO, Haroldson MA, Gunther KA, Phillips DL, Robbins CT. 2003.** Use of sulfur and nitrogen stable isotopes to determine the importance of whitebark pine nuts to Yellowstone grizzly bears. *Canadian Journal of Zoology* **81**:763–770 DOI [10.1139/z03-054](https://doi.org/10.1139/z03-054).
- Hermesen E, Kerle A, Old JM. 2016.** Diet of an inland population of the common ringtail possum (*Pseudocheirus peregrinus*). *Australian Mammalogy* **38**:130–134 DOI [10.1071/AM15008](https://doi.org/10.1071/AM15008).
- Hilderbr GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C. 1996.** Use of stable isotopes to determine diets of living and extinct bears. *Canadian Journal of Zoology* **74**:2080–2088 DOI [10.1139/z96-236](https://doi.org/10.1139/z96-236).
- Huntly N, Inouye R. 1988.** Pocket gophers in ecosystems: patterns and mechanisms. *BioScience* **38**:786–793 DOI [10.2307/1310788](https://doi.org/10.2307/1310788).
- Hwang T, Millar S, Longstaffe J. 2007.** Do $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of feces reflect the isotopic composition of diets in small mammals? *Canadian Journal of Zoology* **85**:388–396 DOI [10.1139/Z07-019](https://doi.org/10.1139/Z07-019).
- Kartzinel TR, Chen PA, Coverdale TC, Erickson DL, Kress WJ, Kuzmina ML, Rubenstein DI, Wang W, Pringle RM. 2015.** DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Science of the United States of America* **112**:8019–8024 DOI [10.1073/pnas.1503283112](https://doi.org/10.1073/pnas.1503283112).
- Kincaid WB, Cameron GN. 1982.** Dietary variation in three sympatric rodents on the Texas Coastal Prairie. *Journal of Mammalogy* **63**:668–672 DOI [10.2307/1380277](https://doi.org/10.2307/1380277).
- Macko SA, Fogel ML, Hare PE, Hoering TC. 1987.** Isotopic fractionation of nitrogen and carbon in the synthesis of amino acids by microorganisms. *Chemical Geology: Isotope Geoscience* **65**:79–92 DOI [10.1016/0168-9622\(87\)90064-9](https://doi.org/10.1016/0168-9622(87)90064-9).
- Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ. 2009.** Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* **84**:91–111 DOI [10.1111/j.1469-185X.2008.00064.x](https://doi.org/10.1111/j.1469-185X.2008.00064.x).
- Melero Y, Palazón S, Bonesi L, Gosalbez J. 2008.** Feeding habits of three sympatric mammals in NE Spain: the American mink, the spotted genet, and the Eurasian otter. *Acta Theriologica* **53**:263–273 DOI [10.1007/BF03193123](https://doi.org/10.1007/BF03193123).

- Montanari S, Amato G. 2015.** Discrimination factors of carbon and nitrogen stable isotopes from diet to hair and scat in captive tigers (*Panthera tigris*) and snow leopards (*Uncia uncia*). *Rapid Communications in Mass Spectrometry* **29**:1062–1068 DOI [10.1002/rcm.7194](https://doi.org/10.1002/rcm.7194).
- Pearson SF, Levey DJ, Greenberg CH, Martínez Del Rio C. 2003.** Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* **135**:516–523 DOI [10.1007/s00442-003-1221-8](https://doi.org/10.1007/s00442-003-1221-8).
- Pecquerie L, Nisbet RM, Fablet R, Lorrain A, Kooijman SA. 2010.** The impact of metabolism on stable isotope dynamics: a theoretical framework. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**:3455–3468 DOI [10.1098/rstb.2010.0097](https://doi.org/10.1098/rstb.2010.0097).
- Pompanon F, Deagle BE, Symondson WO, Brown DS, Jarman SN, Taberlet P. 2012.** Who is eating what: diet assessment using next generation sequencing. *Molecular Ecology* **21**:1931–1950 DOI [10.1111/j.1365-294X.2011.05403.x](https://doi.org/10.1111/j.1365-294X.2011.05403.x).
- Post DM. 2002.** Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**:703–718 DOI [10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2).
- R Core Team. 2016.** R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at <https://www.r-project.org/>.
- Reitsema LJ. 2012.** Introducing fecal stable isotope analysis in primate weaning studies. *American Journal of Primatology* **74**:926–939 DOI [10.1002/ajp.22045](https://doi.org/10.1002/ajp.22045).
- Rodgers TW, Janečka JE. 2013.** Applications and techniques for non-invasive faecal genetics research in felid conservation. *European Journal of Wildlife Research* **59**:1–16 DOI [10.1007/s10344-012-0675-6](https://doi.org/10.1007/s10344-012-0675-6).
- Royer A, Queffelec A, Charlier K, Puech E, Malaizé B, Lenoble A. 2015.** Seasonal changes in stable carbon and nitrogen isotope compositions of bat guano (Guadeloupe). *Palaeogeography, Palaeoclimatology, Palaeoecology* **440**:524–532 DOI [10.1016/j.palaeo.2015.09.033](https://doi.org/10.1016/j.palaeo.2015.09.033).
- Salvarina I, Yohannes E, Siemers BM, Koselj K. 2013.** Advantages of using fecal samples for stable isotope analysis in bats: evidence from a triple isotopic experiment. *Rapid Communications in Mass Spectrometry* **27**:1945–1953 DOI [10.1002/rcm.6649](https://doi.org/10.1002/rcm.6649).
- Sare DT, Millar JS, Longstaffe FJ. 2005.** Tracing dietary protein in red-backed voles (*Clethrionomys gapperi*) using stable isotopes of nitrogen and carbon. *Canadian Journal of Zoology* **83**:717–725 DOI [10.1139/z05-064](https://doi.org/10.1139/z05-064).
- Shehzad W, McCarthy TM, Pompanon F, Purevjav L, Coissac E, Riaz T, Taberlet P. 2012.** Prey preference of snow leopard (*Panthera uncia*) in South Gobi, Mongolia. *PLOS ONE* **7**:e32104 DOI [10.1371/journal.pone.0032104](https://doi.org/10.1371/journal.pone.0032104).
- Sponheimer M, Robinson T, Ayliffe L, Passey B, Roeder B, Shipley L, Lopez E, Cerling T, Dearing D, Ehleringer J. 2003a.** An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. *Canadian Journal of Zoology* **81**:871–876 DOI [10.1139/z03-066](https://doi.org/10.1139/z03-066).
- Sponheimer M, Robinson TF, Roeder BL, Passey BH, Ayliffe LK, Cerling TE, Dearing MD, Ehleringer JR. 2003b.** An experimental study of nitrogen flux in llamas:

is ^{14}N preferentially excreted?. *Journal of Archaeological Science* **30**:1649–1655
[DOI 10.1016/S0305-4403\(03\)00066-9](https://doi.org/10.1016/S0305-4403(03)00066-9).

Steele KW, Daniel RM. 1978. Fractionation of nitrogen isotopes by animals: a further complication to the use of variations in the natural abundance of ^{15}N for tracer studies. *The Journal of Agricultural Science* **90**:7–9 [DOI 10.1017/S002185960004853X](https://doi.org/10.1017/S002185960004853X).

Sutoh M, Koyama T, Yoneyama T. 1987. Variations of natural ^{15}N abundances in the tissues and digesta of domestic animals. *Radioisotopes* **36**:74–77.

Sutoh M, Obara Y, Yoneyama T. 1993. The effects of feeding regimen and dietary sucrose supplementation on natural abundance of ^{15}N in some components of ruminal fluid and plasma of sheep. *Journal of Animal Science* **71**:226–231
[DOI 10.2527/1993.711226x](https://doi.org/10.2527/1993.711226x).

Terry RC. 2010. The dead do not lie: using skeletal remains for rapid assessment of historical small-mammal community baselines. *Proceedings of the Royal Society of London B: Biological Sciences* **277**:1193–1201 [DOI 10.1098/rspb.2009.1984](https://doi.org/10.1098/rspb.2009.1984).

Wickham H. 2009. *ggplot2: elegant graphics for data analysis*. New York: Springer.