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Vicky Oelze, Alice Percher, Gontran Nsi Akoué, Nory El Ksabi, Eric Willaume, Marie Charpentier

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Seasonality and inter-individual variation in mandrill feeding ecology revealed by stable isotope analyses of hair and blood

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7 2 **revealed by stable isotope analyses of hair and blood**
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13 4 Vicky M Oelze^{1,2}, Alice M Percher³, Gontran Nsi Akoué⁴, Nory El Ksabi⁵, Eric
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15 5 Willaume⁵, Marie JE Charpentier^{3*}
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17
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20
21
22 7 ¹ Anthropology Department, University of California Santa Cruz, Santa Cruz, USA

23
24 8 ² Department of Primatology, Max Planck Institute for Evolutionary Anthropology,
25
26 9 Leipzig, Germany

27
28 10 ³ Institut des Sciences de l'Évolution de Montpellier UMR5554, CNRS, IRD, EPHE,
29
30 11 Université de Montpellier, Montpellier, France

31
32 12 ⁴ Université des Sciences et Techniques de Masuku, Franceville, Gabon

33
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35 13 ⁵ SODEPAL, Bakoumba, Gabon
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40 15 **Running head:**

41
42 16 Stable isotope analyses in free ranging mandrills
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47
48 18 ***Corresponding author:**

49 19 Marie JE Charpentier

50 20 ISEM UMR5554

51 21 Place Eugène Bataillon cc065

52 22 34095 Montpellier cedex 05, France

53 23 Email: marie.charpentier@umontpellier.fr
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4 **Abstract**
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7 26 Mandrills are large bodied, terrestrial forest primates living in particularly large social
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9 27 groups of several hundred individuals. Following these groups in the wild to assess
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11 28 differences in diet over time as well as among individuals is demanding. We here use
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13 29 isotope analyses in blood and hair obtained during repeated captures of 43 identified
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15 30 free-ranging mandrills (*Mandrillus sphinx*) from Southern Gabon, to test how dietary
16
17 31 variation relates to season as well as an individual's age and sex. We measured the
18
19 32 stable carbon ($\delta^{13}\text{C}\%$) and nitrogen ($\delta^{15}\text{N}\%$) isotope ratios in 46 blood and 214 hair
20
21 33 section samples as well as from a small selection of mandrill foods (n=24). We found
22
23 34 some seasonal isotopic effects, with lower $\delta^{13}\text{C}$ values but higher $\delta^{15}\text{N}$ values observed
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25 35 during the highly competitive long dry season compared to the fruit-rich long rainy
26
27 36 season. Variation in $\delta^{13}\text{C}$ was further predicted by individual age, with higher $\delta^{13}\text{C}$
28
29 37 values generally found in younger individuals suggesting that they may consume more
30
31 38 high canopy fruit than older individuals, or that older individuals consume more low
32
33 39 canopy foliage. The best predictor for $\delta^{15}\text{N}$ values was the interaction between age and
34
35 40 sex, with mature and reproductively active males revealing the highest $\delta^{15}\text{N}$ values,
36
37 41 despite the observation that males consume substantially less animal food items than
38
39 42 females. We interpret high $\delta^{15}\text{N}$ values in these mature male mandrill blood and hair
40
41 43 sections to be the result of nutritional stress associated to intense male-male
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43 44 competition, particularly during mating season. This is the first study showing isotopic
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45 45 evidence for nutritional stress in a free-ranging primate species and may spark further
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47 46 investigations into male mandrill diet and energy balance.
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49 **Keywords:** carbon, nitrogen, sex differences, season
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49 **Research Highlights**

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51 • Stable isotope analyses of hair and blood have the potential to provide short and
52 long-term data on how individual diets may shift over the season and with
53 individual traits.

54 • Dietary patterns differ across seasons, between sexes as well as between young
55 and older mandrills.

56 • Mature and reproductively active male mandrills showed the highest nitrogen
57 isotope values as a result of nutritional stress possibly associated to intense
58 male-male competition in this species.

59 • This is the first study showing isotopic evidence for nutritional stress in a free-
60 ranging primate species.

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62 **Introduction**

63 Feeding ecology studies are essential to determine an individuals' nutritional and
64 energetic requirements and understand how they translate into different biological and
65 ecological characteristics of a species, such as ranging patterns and socio-sexual
66 behavior (e.g. Chapman and Chapman, 1990, Hohmann et al., 2006, Lambert and
67 Rothman, 2015; McGraw and Daegling, 2012). As with many other animal species,
68 primate sociality highly depends on how food is distributed in the environment. The
69 degree at which individuals use clumped and high energy food resources such as fruits
70 vs. much more dispersed lower energy foods such as foliage gives insights into the
71 evolution of sociality across primate species (Clutton-Brock and Janson, 2012; van
72 Schaik, 1989; Sterck et al., 1997).

73 Compared to other terrestrial large bodied primates in Africa, such as great apes and
74 baboons, mandrills (*Mandrillus sphinx*) are clearly understudied in the wild. Our
75 knowledge about mandrill feeding seasonality and competition is limited to a handful of
76 studies conducted in the 1980s (Harrison, 1988; Hoshino et al., 1984; Lahm, 1986;
77 Rogers et al., 1996) as well as much more recent work at a research site in Southern
78 Gabon, the Lékédi Park (Nsi Akoué et al., 2017). Our current understanding is that
79 mandrills are generalist feeders with a clear tendency towards frugivory (Lahm, 1986;
80 Nsi Akoué et al., 2017). During a 17-month behavioral survey, 57 mandrills from the
81 Lékédi population were observed consuming about 150 different plant species
82 (including more than 440 different items), representing 74% of their diet, along with
83 mushrooms (<1%), invertebrates (6%) and vertebrates (<1%) (Nsi Akoué et al., 2017).
84 Behavioral data additionally suggest that females consumed more faunal food items
85 than males (Nsi Akoué et al., 2017). Among the plant species frequently consumed,

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86 mandrills relied essentially on C₃ plants (e.g. *Xylopia aethiopica* and *Pentaclethra*
87 *macrophylla*), including bamboo, a C₃ plant within the grass family *Poaceae* (e.g. *Olyra*
88 *latifolia*, see Nsi Akoué et al., 2017).

89 While long-term and direct observations of feeding behavior generally constitute the
90 ideal method for determining dietary differences between individuals and across
91 seasons, this requires full habituation and long-term systematic study. In mandrills,
92 direct observations are partially impeded by the fairly large group sizes of several
93 hundred individuals (Abernethy et al., 2002; Harrison, 1988; Rogers et al., 1996). The
94 use of indirect methods to study primate diets are thus a tempting and cost-effective
95 alternative to some, clearly not all, aspects of direct observation. Fecal analysis provides
96 a good overview of what food items are eaten by a primate species over short to long
97 term periods and can be conducted even in unhabituated groups (e.g. Tutin and
98 Fernandez, 1993). However, while fecal analysis is strictly non-invasive and reveals
99 some aspects of what was ingested by the animals the previous day(s), the dietary
100 assessment is potentially biased towards indigestible food items such as fruit seeds,
101 tough foliage and insect chitin (Lahm, 1986) and the method requires repeated sampling
102 over large stretches of time. As an alternative approach, the stable isotopes of carbon
103 ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) are increasingly used to reconstruct diet and ecological
104 niches of wild animal species with less sampling effort (Crowley, 2012; Crowley et al.,
105 2015). For example, patterns of seasonal dietary overlap and dietary differentiation
106 found in isotope values of hair collected non-invasively from nests of central
107 chimpanzees (*Pan troglodytes troglodytes*) and lowland gorillas (*Gorilla gorilla gorilla*)
108 suggested plasticity in the feeding niches of these sympatric apes (Oelze et al., 2014),

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4 109 consistent with previous results based on long-term and labor intensive fecal sample
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6 110 collection and analysis (Head et al., 2011).
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9 111 Stable isotope analysis in samples of body tissue provides estimates of several aspects
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11 112 of the animals' diet in the recent or more distant past (Crowley, 2012; Crowley et al.,
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13 113 2015; Sandberg et al., 2012). Feces and blood are for example turned over by the body
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15 114 fairly rapidly and are related to the diet of the previous days or weeks (Hobson and
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17 115 Clark, 1992). By contrast, hair keratin has a much slower turnover rate of ~1cm per
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19 116 month (Tobin, 2005; Fourie et al., 2016), although hair growth rates are yet unknown
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21 117 for most wild primates necessitating to fall back on human hair growth rates as a proxy
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23 118 (Oelze, 2016). In hair, sampling along the growth trajectory from hair root to tip allows
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25 119 for a isotopic time sequence of several consecutive months (O'Connell and Hedges,
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27 120 1999; Schwertl et al., 2003; Oelze, 2016). The use of sequential hair isotope analysis
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29 121 has shown to be particularly useful to investigate dietary changes over time in response
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31 122 to shifts in climate and food availability as well as dietary differences between
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33 123 individuals within the same social group (Oelze, 2016; Oelze et al., 2011, 2014, 2016a).
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39 124 Some aspects of diet may be reconstructed using stable isotopes because the ratios of
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41 125 the stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C} = \delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N} = \delta^{15}\text{N}$) in animal
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43 126 bodies are sourced from the predominant foods, which can vary drastically and thus be
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45 127 relevant dietary markers (DeNiro and Epstein, 1978, 1981). On the one hand, the $\delta^{13}\text{C}$
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47 128 values of plants differ substantially between C_3 and C_4 photosynthetic pathways
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49 129 (Tieszen, 1991), but also between high canopy and low canopy plant resources in forest
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51 130 habitats (Medina and Minchin, 1980; van der Merwe and Medina, 1989), and even
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53 131 between photosynthetic (e.g. foliage) and reproductive plant parts (e.g. fruits and
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55 132 flowers; Cernusak et al., 2009). In C_3 plant dominated tropical rainforest habitats, we
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4 133 can expect the majority of variation in $\delta^{13}\text{C}$ in herbivore consumers to be associated
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6 134 with differences in the consumption of high versus low canopy food items (e.g. arboreal
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9 135 fruit versus terrestrial herbs), as well as between reproductive and photosynthetic plant
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11 136 parts (e.g. fruit versus leaves; Blumenthal et al., 2016; Oelze et al., 2016a; Roberts et
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13 137 al., 2017). On the other hand, the $\delta^{15}\text{N}$ values in foods mainly relate to the trophic level
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15 138 of a food item, with plants revealing the lowest $\delta^{15}\text{N}$ values (DeNiro and Epstein, 1981).
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18 139 Primary consumers have significantly higher $\delta^{15}\text{N}$ values than the plants they eat,
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20 140 whereas carnivores reveal higher $\delta^{15}\text{N}$ values than herbivores (DeNiro and Epstein,
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22 141 1981).

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25 142 While differences between some food categories can be striking, there are several
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27 143 environmental factors that influence the isotopic variation within plants in a given
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29 144 habitat, including temperature (Barnes et al., 2007), rainfall (Nardoto et al., 2008; Sealy
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31 145 et al., 1987), altitude (Tieszen et al., 1979), soil composition (Dawson et al., 2002), as
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33 146 well as canopy density (Medina and Minchin, 1980). One strategy to cope with these
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35 147 environmental influences is to assess the local isotope baseline using a small yet
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37 148 representative plant sample, ideally consisting of plant foods frequently consumed by
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39 149 the study subjects (Oelze et al., 2011, 2016b). There is no widespread consensus on how
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41 150 extensive this plant sample should be to represent the habitat, and some studies still do
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43 151 not include any plant samples at all (Loudon et al., 2014; Schoeninger et al., 2015;
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45 152 Sponheimer et al., 2006). However, while these baseline values are crucial when
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47 153 comparing between primate groups ranging in different habitats with different
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49 154 environmental conditions (Oelze et al., 2016b), they may be less essential to studies
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51 155 focusing on intra-community differences as well as isotopic variation over time.
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4 156 Isotopic patterns can vary considerably between individuals and reveal information on
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6 157 their access to food as well as on their physiological condition. For example, sex and/or
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9 158 social rank can result in a biased access to specific desirable foods which can be distinct
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11 159 in their $\delta^{15}\text{N}$ values, e.g. vertebrate meat (Fahy et al., 2013; Oelze et al., 2011, 2016a).
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13 160 Also the energetic demands of female reproduction (gestation, lactation) can affect her
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15 161 bodies nitrogen balance and thus skew the $\delta^{15}\text{N}$ values (Fuller et al., 2004; Oelze et al.,
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17 162 2016a). Further, there is good evidence that negative energy balance due to severe
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19 163 nutritional stress or fasting can result in shifts in nitrogen metabolism and thus $\delta^{15}\text{N}$
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21 164 values in primates (Deschner et al., 2012; Mekota et al., 2006) and other animals
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23 165 (Hobson et al., 1993).
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28 166 In this study, we evaluate to what extent stable isotope analysis can provide novel
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30 167 information regarding the feeding ecology of a natural population of mandrills living at
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32 168 the Lékédi Park and surroundings, in Southern Gabon. In particular, we focus on the
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34 169 effects of shifting environmental conditions (season) and individual characteristics (age,
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36 170 sex) on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured in mandrill body tissue samples. We here
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38 171 use two different sample tissues with two very different turnover rates and thus
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40 172 reflecting different periods in time. Blood isotope data is considered here to reflect the
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42 173 recent dietary contributions of the past day(s) with relatively high accuracy, whereas
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44 174 hair section isotope data relates to the isotopic characteristics of foods consumed over
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46 175 the previous months, with data representing weekly or monthly intervals. We assess to
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48 176 what extent these two different sample types pick up the same isotopic patterns.
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53 177 Given the previous isotopic research in tropical forest of Gabon (Oelze et al., 2014), as
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55 178 well as previous observational data on this primate population (Nsi Akoue et al., 2017),
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57 179 we focus on the following predictions. Regarding the season of sampling, we expect
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4 180 isotopic values to vary between rainy and dry seasons, as fruit availability is commonly
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6 181 shifting with rainfall. Shifting proportions of foliage vs. fruit should particularly have an
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8 182 effect on the $\delta^{13}\text{C}$ values of mandrills, with high fruit intake periods being associated
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10 183 with high $\delta^{13}\text{C}$ values (Blumenthal et al., 2016; Cernusak et al., 2009; Oelze et al.,
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12 184 2016a; Roberts et al., 2017). Regarding individual age, we predict that the intense intra-
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14 185 sexual competition observed in fully adult males (Setchell et al., 2005), as compared to
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16 186 younger, non-reproductive males, may result in nutritional stress particularly during the
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18 187 mating season (long dry season) with higher $\delta^{15}\text{N}$ values in mature males. Finally,
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20 188 regarding individual sex, we expect to find slightly higher $\delta^{15}\text{N}$ values in all females as
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22 189 compared to all males as females have been shown to consume more animal food items
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24 190 than males (Nsi Akoué et al., 2017), which can be expected to have higher $\delta^{15}\text{N}$ values
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26 191 than plant foods.
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193 **Materials and Methods**

194 *Ethical statement*

195 This study complies with ethical protocols approved by the CENAREST institution
196 (authorization number: AR003/20/MESRSTT/CENAREST/CG/CST/CSAR). This
197 research adhered to the legal requirements of Gabon and to the American Society of
198 Primatologists principles for the ethical treatment of nonhuman primates.

199 *Site and study population*

200 The study group originates from 65 captive individuals released into the park on two
201 occasions in 2002 and 2006 (Peignot et al., 2008). Food supplementation with bananas
202 and home-made cakes three to five times a week was initiated with the release, then

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4 203 gradually declined for a few years and totally ceased in April 2012 except during
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6 204 punctual trappings. The study population is monitored daily within the framework of a
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9 205 long-term field project (Mandrillus Project) started in early 2012. In 2018, the group
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11 206 was composed of ca. 180-200 habituated and individually identified mandrills, of which
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13 207 ~ 90% were born in the wild. We collected tissue samples from a total of 43 individuals
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15 208 (24 males, 19 females) aged 2.2-19.3 yrs. Data on individual age in this study are based
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17 209 on observed birth dates (n=12) or are based on general body conditions as well as tooth
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19 210 eruption and wear patterns (Galbany et al., 2014).

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23 211 The study population ranges freely in the Lékédi Park and its surroundings near the
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25 212 village of Bakoumba. This private park covers an area of 116km² at an elevation
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27 213 varying from 430 to 610m. The habitat is composed of closed canopy forests and
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29 214 patches of savanna (Brockmeyer et al., 2015). Gabon is characterized by an equatorial
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31 215 climate with a long rainy season (Feb-May; in the studied site: monthly average
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33 216 temperature=23.9°C and total rainfall=240.5 mm; data pooled from 04/2012-12/2014), a
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35 217 long dry season (Jun-Sep; 22.1°C and 18.9 mm), a short rainy season (Oct-Nov; 23.3°C
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37 218 and 152.3 mm) and a short dry season (Dec-Jan; 23.5°C and 133.3 mm). These four
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39 219 climatic seasons have been shown to result in distinct diets in the studied mandrills,
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41 220 with no overlap in food selection between these seasons (see Figure 1 in: Nsi Akoué et
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43 221 al., 2017). In addition, reproduction is highly seasonal in mandrills, with most females
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45 222 cycling during the long dry season and giving birth during the short dry season or at the
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47 223 beginning of the long rainy season (MJEC, pers. obs). We therefore considered these
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49 224 four climatic seasons as a reliable indicator for the seasonality of mandrill behavior and
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51 225 ecology.

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58 226 *Sample collection*
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4 227 We collected hair tufts and blood samples from mandrills during four capture events
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6 228 between April 2012 and July 2014. During these events, mandrills were baited with
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9 229 food (mostly bananas) and anesthetized via blowpipe intramuscular injection of a
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11 230 ketamine-xylazine mix. After 30-40 min, individuals were awakened using atipamezole.
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13 231 During anesthesia, we plucked hair from the mandrills' arms or legs and stored them in
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15 232 paper envelopes that were then stored dry. We collected blood samples from the iliac
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17 233 vein of the individuals using heparinized tubes. Blood samples were centrifuged (15
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19 234 min at 3000 rpm) the day of collection to obtain blood samples that were stored at -
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21 235 20°C (Beaulieu et al., 2017).

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24 236 In 2014, we collected 24 food samples consumed by the studied mandrills, including 14
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26 237 plant species, three mushroom and three insect species (Table S1). These items were
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28 238 selected randomly among the hundreds of food items known to be consumed by the
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30 239 studied population to gain a broad impression on the plant baseline values in the habitat,
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32 240 without an expectation of grasping the entire picture of plant isotope diversity. All
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34 241 selected plant species are frequently consumed by the studied mandrills (>0.10
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36 242 occurrence per hour, as defined per: Nsi Akoué et al., 2017).

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39 243 We prepared 214 hair sections from 62 hair samples. We selected 3 to 12 hairs of
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41 244 similar length, diameter and growth stage from each hair tuft and followed a sequential
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43 245 sectioning protocol (see for details: Oelze, 2016). Each hair sample provided up to 12
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45 246 different sections of 1cm (3.5 on average), which we sometimes combined to reach a
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47 247 weight suitable for a single isotopic measurement. We aliquoted 200 µL of 46 blood
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49 248 samples and freeze-dried these samples for 24h before analyses. Plants were dried the
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51 249 day of collection and homogenized thereafter. Insects and mushrooms were frozen at -
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4 250 20°C the day of collection, freeze-dried during 24h and then homogenized using a
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6 251 pebble mill before analyses.

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9 252 Hair sections (0.33 to 0.74 mg), blood (0.88 to 1.14 mg) and food samples (0.44 to
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11 253 2.25mg) were weighed into tin capsules for isotopic measurements performed parallel
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14 254 to the IAEA standards CH6, CH7, N1 and N2 in a FLASH HT Plus coupled to a MAT
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16 255 253 IRMS (both by Thermo Scientific, Waltham, MA, USA) at the commercial
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18 256 laboratory IsoDetect GmbH in Leipzig, Germany. Stable isotope ratios are expressed in
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21 257 permil (‰) using the delta (δ) notation and are calibrated against the international
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23 258 standard materials Vienna PeeDee Belemite (vPDB) for carbon and atmospheric N₂
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25 259 (AIR) for nitrogen. Analytical error calculated from repetitive measurements of
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28 260 international and lab-internal standard materials in each run is less than 0.2‰ (2 σ) for
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30 261 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Stable isotope ratios for hair sections, blood samples and food items are
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32 262 hereafter referred to as $\delta^{13}\text{C}_{\text{hair}}$, $\delta^{13}\text{C}_{\text{blood}}$, and $\delta^{13}\text{C}_{\text{diet}}$ as well as $\delta^{15}\text{N}_{\text{hair}}$, $\delta^{15}\text{N}_{\text{blood}}$, and
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34 263 $\delta^{15}\text{N}_{\text{diet}}$. We removed one blood sample from the dataset because of its abnormally high
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36 264 value of C:N ratio (5.10 against an average value=3.56±0.08).

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40 265 *Estimation of diet-tissue fractionation factors*

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42 266 Despite the small sample set we have for mandrill plant food items, we calculated
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44 267 isotopic fractionation factors for both isotope systems ($\Delta^{15}\text{N}_{\text{hair-diet}}$ and $\Delta^{13}\text{C}_{\text{hair-diet}}$)
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46 268 between broad categories of plant foods (reproductive parts vs. non-reproductive parts;
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48 269 see: Table S1) and individual hair sections to obtain a broad impression of which plant
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50 270 food category is relevant to mandrill diet and is reflected in hair isotope values. In
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52 271 addition, this approach controls for baseline differences and allows to compare between
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54 272 populations and sites with differing environmental conditions (Crowley et al., 2013;
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56 273 Nakagawa et al., 2007; Oelze et al., 2016b).

274 *Statistical analyses*

275 We conducted all statistical analyses in SAS Studio. To test for relationships between
276 isotopic values obtained from hair and blood samples representing the same individual
277 and the same time window, we used Pearson correlation tests. We used Linear Mixed
278 Models (LMM, proc GLIMMIX) and Linear Models (LM, proc GLM) with Gaussian
279 distributions to study the effects of seasonality and individual traits on both $\delta^{13}\text{C}$ and
280 $\delta^{15}\text{N}$ recorded in hair sections (LMM) and blood samples (LM). The normality of the
281 models' residuals was systematically verified by visual inspection of the residuals'
282 distribution in qq-plots and by conducting Kolmogorov-Smirnov tests. Note that in one
283 model ($\delta^{15}\text{N}_{\text{hair}}$), we removed six outlier samples to attain a Gaussian distribution of the
284 data (leverage test, proc robustreg). We used differences in least square means as post-
285 hoc analyses for pairwise comparisons of categorical variables when appropriate. In all
286 our statistical models performed, we considered all first order interactions and kept the
287 full models as final models excluding only non-significant interactions ($p>0.05$).

288 We ran four independent models on the response variables $\delta^{13}\text{C}_{\text{hair}}$, $\delta^{15}\text{N}_{\text{hair}}$, $\delta^{13}\text{C}_{\text{blood}}$
289 and $\delta^{15}\text{N}_{\text{blood}}$ to explore the effects of season, individual age and sex. We added the
290 factor hair sample as a random effect in the two models on hair isotope values to control
291 for pseudo-replication biases caused by multiple sampling of the hair tufts (Mundry and
292 Oelze, 2015). We did not consider individual as a random effect in the analyses based
293 on blood samples because only few animals out of our dataset were sampled more than
294 once ($n=10$).

295 We were unable to estimate mandrill hair growth rates, so we relied on human scalp hair
296 growth rates as a rough estimate (see discussion in: Oelze, 2016). Hence, for each hair
297 section analyzed, we calculated the corresponding time window based on a growth rate

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4 298 of 1 cm of hair per 30 days (Tobin, 2005), with the root date identical to the hair
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6 299 collection date and the distal date corresponding to 30 days before.
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9 300 In all four models we considered sex as a class variable and age as a continuous variable
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11 301 (calculated at the distal date for hair and at the date of collection for blood). We
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13 302 considered the effect of season as a class variable with two (blood) to three (hair)
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15 303 modalities including a long rainy season, a long dry season, and the two short seasons
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17 304 that we pooled together for the models based on hair because of limited sample sizes (7
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19 305 samples only were collected during the small rainy season). In the models based on
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21 306 blood, we considered the season at the exact date of sampling while in the models based
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23 307 on hair, we considered the season at the distal date.
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28 308 Food supplementation that occurred just before the beginning of sampling (early 2012)
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30 309 may have impacted isotope values in the distal sections of long mandrills' hair (the ones
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32 310 spanning the supplementation period that ceased in April 2012). Although
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34 311 supplementation only constituted a limited proportion of mandrills' diet, we conducted
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36 312 preliminary analyses by removing these potentially biased hair sections. We found
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38 313 similar results to those obtained from full datasets and therefore chose to include these
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40 314 samples in the final models.
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46 47 48 316 **Results**

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51 317 We obtained a total of 46 isotopic measurements for blood and 214 measurements in
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53 318 hair sections. The $\delta^{13}\text{C}_{\text{hair}}$ values range from -26.1‰ to -24.0‰ (mean $\pm 1\sigma$: $-25.1 \pm$
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55 319 0.3%), slightly higher than $\delta^{13}\text{C}_{\text{blood}}$ values which vary between -27.7‰ and -25.8‰ ($-$
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57 320 $26.6 \pm 0.4\%$). The $\delta^{15}\text{N}_{\text{hair}}$ values range from 4.21‰ to 7.8‰ ($6.0 \pm 0.5\%$) while
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4 321 $\delta^{15}\text{N}_{\text{blood}}$ values vary less and range between 5.6‰ and 7.5‰ ($6.4 \pm 0.3\%$). Isotopic
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6 322 measurements retrieved from hair sections and blood samples collected from the same
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8 323 individual on the same day ($n=37$) are significantly correlated (Figure S1). Despite the
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10 324 small plant food dataset from the studied habitat, we can report that non-reproductive
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12 325 plant parts ($-32.2 \pm 3.0\%$) yielded on average $\sim 2\%$ lower $\delta^{13}\text{C}$ values than reproductive
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14 326 plant parts ($-30.3 \pm 2.6\%$). In addition, $\delta^{15}\text{N}$ values were on average higher in non-
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16 327 reproductive plant parts ($6.0 \pm 3.8\%$) than in reproductive plant parts ($3.7 \pm 1.1\%$). The
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18 328 few arthropods we analyzed (ants, butterfly, caterpillar) did not have higher $\delta^{15}\text{N}$ values
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20 329 than plant foods (Table S1). Mean fractionation factors between mandrill hair and
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22 330 reproductive plant parts, comprised by fruits, seeds and some flowers, resulted in mean
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24 331 $\Delta^{15}\text{N}_{\text{hair-fruit}}$ values of 2.3‰ and mean $\Delta^{13}\text{C}_{\text{hair-fruit}}$ values of 5.2‰. The calculated mean
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26 332 fractionation values between mandrill hair and non-reproductive plant parts (leaves)
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28 333 were 0.0‰ for $\Delta^{15}\text{N}_{\text{hair-leaves}}$ and 7.1‰ for $\Delta^{13}\text{C}_{\text{hair-leaves}}$.
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334 335 *Effect of season*

336 In the $\delta^{13}\text{C}_{\text{blood}}$ model, isotopic values were significantly higher during the rainy season
337 (see Table 1 for detailed statistical results; Figure 1A). In the $\delta^{13}\text{C}_{\text{hair}}$ model, however,
338 the factor season was interacting with the predictors sex and age (Table 1). In females,
339 the lowest $\delta^{13}\text{C}$ values of hair were found during the long rainy season, while we
340 observed exactly the reverse in males (Figure 2). Although the interaction between age
341 and season was not obvious, younger animals, especially males, tended to show higher
342 $\delta^{13}\text{C}$ values of hair (Figure 2). The $\delta^{15}\text{N}$ values of blood were significantly higher
343 during the long dry season compared to the long rainy season (Table 1; Figure 1B). By
344 contrast, the effect of seasonality was not captured by the $\delta^{15}\text{N}$ values of hair (Table 1).

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7 346 *Effects of individual age and sex*

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9 347 Individual age had a significant effect on $\delta^{13}\text{C}$ values of blood which decreased with
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11 348 mandrill's age in male subjects (Table 1; Figure 3). Finally, we found significant
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13 349 interactions between individual age and sex on $\delta^{15}\text{N}$ values of blood and hair: older
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15 350 males showed higher values than younger males and this age effect was not observed in
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18 351 females (Table 1; Figure 4A and B).

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24 353 **Discussion**

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27 354 We here report the first isotopic study conducted on free ranging mandrills in which we
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29 355 investigated effects of season and individual traits such as age and sex on feeding
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31 356 behavior. The limited plant isotope data set allowed a glimpse into the natural isotopic
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33 357 variation of food items in the mandrill habitat to understand how to interpret findings of
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35 358 lower or higher isotope values across seasons and individuals. Similar to isotopic values
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37 359 reported in several forests of Central and West African countries (Oelze et al., 2014;
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39 360 Oelze et al., 2016b), the low $\delta^{13}\text{C}$ values of the 14 plant species collected in the Lékédi
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41 361 Park (mean: -31.3‰) indicate that mandrills' habitat is similarly depleted in ^{13}C as other
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43 362 forest habitats in Gabon, such as Loango with a mean plant $\delta^{13}\text{C}$ value of -30.5‰
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45 363 (Oelze et al., 2014), although additional plant isotope data would be desirable to
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47 364 confirm this finding.

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53 365 The $\delta^{13}\text{C}$ values of mandrills' hair sections ($-25.1\pm 0.3\text{‰}$) are within the range of those
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55 366 obtained from hair of sympatric great apes also living in Gabon, namely central
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57 367 chimpanzees (from -25.7‰ to -23.4‰) and lowland gorillas (from -27.1‰ to -24.5‰)
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4 368 from Loango National Park (Oelze et al., 2014). Similar to these species, mandrills live
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6 369 in dense rainforest habitats strongly affected by the so called canopy effect (van der
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9 370 Merwe and Medina, 1989). As expected, mandrills yielded much lower $\delta^{13}\text{C}$ values than
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11 371 those found in South African chacma baboons (ranging from -19.8‰ to -18.5‰) as
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13 372 these baboons live in an open semiarid environment and ~20-30% of their diet is
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15 373 comprised by C_4 plants (Codron et al., 2006). Mandrills showed on average higher
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17 374 $\delta^{15}\text{N}_{\text{hair}}$ values ($6.0 \pm 0.4\text{‰}$) than chimpanzees and gorillas from Gabon (5.0‰ and 4.2‰,
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19 375 resp.), which is well explained by the slightly lower mean $\delta^{15}\text{N}$ value in plants from
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21 376 Loango (4.2 at Lékédi vs. 3.3‰ at Loango; Oelze et al., 2014). These similarities with
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23 377 other species in both isotope systems would suggest that the dietary pattern observed in
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25 378 mandrills is comparable to the dietary niche of terrestrial great apes in other parts of
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27 379 Gabon, particularly to the higher level of frugivory as seen in chimpanzees. While our
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29 380 food item isotope dataset was too small to conduct dietary mixing models, at least the
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31 381 fractionation factors we calculated between different plant categories and mandrills
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33 382 seem to support this finding, as the relationship between mandrill hair and the different
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35 383 plant foods found a better match between reproductive plant parts (fruits, seeds and
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37 384 flowers) than it did with foliage. $\Delta^{13}\text{C}_{\text{hair-fruit}}$ values between ~3 and 5‰ and $\Delta^{15}\text{N}_{\text{hair-fruit}}$
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39 385 values between ~2 and 4‰ are reported for several populations of frugivorous great
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41 386 apes in different regions of Africa (Oelze et al., 2016b). This very general observation is
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43 387 well in line with direct observations of mandrill foraging behavior which suggests that
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45 388 their diet consists of 70 to 90% of fruit (Nsi Akoue et al., 2017). For non-reproductive
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47 389 plant parts (foliage) the fractionation values were either too low (0.0‰ $\Delta^{15}\text{N}_{\text{hair-leaves}}$) or
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49 390 too high (7.1‰ $\Delta^{13}\text{C}_{\text{hair-leaves}}$) to be considered reflecting foliage as a substantial dietary
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51 391 contribution. However, this mismatch could also be related to a data bias introduced by
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4 392 using a very small and possibly not representative sample of leaves, that likely does not
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6 393 encompass the full isotopic variation within non-reproductive plant parts in the
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8 394 mandrills' diet. Particularly leaves can vary substantially in their $\delta^{13}\text{C}$ values depending
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10 395 on whether they are sampled on the forest floor, understory or high up in the canopy
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12 396 (Medina and Minchin, 1980; van der Merwe and Medina, 1991; Carlson and Kingston,
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14 397 2014; Oelze et al., 2014, 2016a; Carlson and Crowley, 2016; Roberts et al., 2017),
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16 398 which may make comparisons of hair-leave values less reliable than calculation
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18 399 referring to reproductive plant parts alone (Oelze et al., 2016b).

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22 400 We found some isotopic differences across seasons. We expected to find
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24 401 isotopic variation in response to shifts in fruit availability between the dry and rainy
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26 402 seasons. In Gabon, the rainy season generally corresponds to higher fruit availability
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28 403 (White, 1994; but see: Head et al., 2011) and high fruit intake periods should be
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30 404 associated with higher $\delta^{13}\text{C}$ values (Cernusak et al., 2009; Oelze et al., 2014, 2016b). As
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32 405 such, we found higher $\delta^{13}\text{C}$ values of blood across all studied mandrills and also higher
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34 406 $\delta^{13}\text{C}$ values of hair in males during the long rainy season compared to the long dry
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36 407 season. It remains unclear if this seasonal pattern in $\delta^{13}\text{C}$ values could be related to
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38 408 general seasonal changes within the $\delta^{13}\text{C}$ values of plant food categories. Previous
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40 409 research on primate forest plant foods did not suggest that seasonal variation is
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42 410 considerable (Carlson and Kingston, 2014) and we thus did not systematically collect
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44 411 samples from the same food plants across different seasons in this study.,

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46 412 The $\delta^{13}\text{C}$ values of hair in females did not follow this seasonal pattern in $\delta^{13}\text{C}$ value,
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48 413 possibly reflecting some sex-related differences in physiology or seasonal feeding
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50 414 preferences. During the long dry season, most females are, indeed, cycling or are in
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52 415 early pregnancy (see Figure 1 in: Dibakou et al., 2019), and the specific nutritional
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4 416 demands of maternal investment have been shown to affect the feeding behavior and
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6 417 $\delta^{13}\text{C}$ values of other large bodied primates (Oelze et al. 2016). The long dry season also
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8 418 caused higher $\delta^{15}\text{N}$ values in blood of the studied mandrills, although this season does
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11 419 not correspond to the highest animal's consumption in the studied population (Nsi
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13 420 Akoué et al., 2017). This apparent contradictory finding probably results from highly
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15 421 shifted $\delta^{15}\text{N}$ values in adult male mandrills (see below).

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18 422 Variation in $\delta^{13}\text{C}$ values of blood was mainly predicted by age but not sex, whereas
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20 423 $\delta^{15}\text{N}$ variation was driven by the interaction between individual age and sex. In blood
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22 424 and, to a lesser extent, in male hair section samples, the $\delta^{13}\text{C}$ values were significantly
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24 425 higher in young individuals compared to old mandrills. Given that variation in plant
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26 426 $\delta^{13}\text{C}$ values is primarily driven by canopy height (lower values in understory than in
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28 427 canopy) and plant part (lower values in foliage than in fruit) in rainforest communities,
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30 428 we can interpret higher $\delta^{13}\text{C}$ values in young mandrills to be the result of consuming
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32 429 significantly less low canopy plant foods and/or foliage, but more fruit from higher in
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34 430 the canopy. Significant diet differences across age classes have been observed during
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36 431 the long dry season in the studied mandrills. At this time of the year, interestingly, the
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38 432 highest frequencies of fruit consumption was observed in juvenile males and adolescent
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40 433 females (see Supplementary Information in: Nsi Akoué et al., 2017). Although, we
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42 434 cannot comment further on this possible relationship, it seems puzzling as one may
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44 435 predict that older, more experienced mandrills should be able to find ripe fruit better
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46 436 than younger individuals. Alternatively, instead of high canopy fruit, the infrequent
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48 437 consumption of C_4 grasses may explain the higher $\delta^{13}\text{C}$ values in young individuals.
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50 438 However, we have little to no evidence that C_4 grasses possibly available in the open
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52 439 savanna patches are of any relevance to the mandrills feeding ecology. Finally, young
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4 440 mandrills were found, among others, to consume significantly more *Ficus mucoso* but
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6 441 less *Aframomum alboviolaceum* than adults in the study population (Nsi Akoué et al.,
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8 442 2017). While fruits of the former should result in high $\delta^{13}\text{C}$ values, because they are
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10 443 generally found high in the canopy, the latter should result in low $\delta^{13}\text{C}$ values because it
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12 444 is a terrestrial herbaceous species. These observations are consistent with our current
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14 445 findings.

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18 446 We anticipated to additionally find another effect of age on the dietary behavior of
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20 447 mandrills as particularly older and reproductively active males may undergo periods of
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22 448 nutritional stress during and around the mating season. An endocrinological study on
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24 449 this mandrill population suggested high fecal glucocorticoid values in old males during
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26 450 the mating season (long dry season) due to the influx of new competing males and
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28 451 intense and aggressive male-male competition in this species (Charpentier et al., 2018).
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30 452 This may suggest that older males undergo negative energy balance for several months
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32 453 a year. Our models and the $\delta^{15}\text{N}$ values obtained from both blood and hair sections
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34 454 appear to support this assumption, with increasing $\delta^{15}\text{N}$ values with age in males. We
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36 455 assume that higher $\delta^{15}\text{N}$ values in older males result from nutritional stress in response
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38 456 to the intense male-male competition during the mating season. Mature male mandrills
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40 457 fiercely fight over access to female and dedicate less time to feeding, resulting in higher
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42 458 stress levels and negative energy balance (Charpentier et al., 2018). Adult males indeed
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44 459 spend less time foraging compared to all other individuals (Nsi Akoué et al., 2017). The
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46 460 $\delta^{15}\text{N}$ values are known to increase in response to severe negative energy balance. When
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48 461 basic metabolic requirements in nitrogen (protein) are not met through diet, it has to be
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50 462 acquired from body own proteins through catabolism. Higher mobilization and
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52 463 excretion of body own nitrogen then contributes to a reduction in body mass (Deschner
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4 464 et al., 2012). The reduction of net energy intake has to be substantial in order to lead to
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6 465 reduction in body mass, and the condition has to be experienced for a prolonged period
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9 466 of time in order to affect the animals physiology, nitrogen balance and in consequence
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11 467 the bodies $\delta^{15}\text{N}$ values (Barboza and Parker, 2006; Hobson et al., 1993). Changes in
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13 468 $\delta^{15}\text{N}$ values due to nutritional stress have rarely been shown in non-human and human
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15 469 primates (Deschner et al., 2012; D'Ortenzio et al., 2015; Mekota et al., 2006, 2009).
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18 470 This is the first study reporting isotopic evidence for nutritional stress in a free-ranging
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20 471 primate supported by observational data, suggesting that nutritional stress experienced
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22 472 by reproductive male mandrills is traceable using isotope analysis and worth future
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25 473 more detailed investigation.

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28 474 Finally, we anticipated to find slightly higher $\delta^{15}\text{N}$ values in all females as compared to
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30 475 all males. As the first direct observations of this population of mandrills suggested that
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32 476 females consumed more animal food items than males (Nsi Akoue et al., 2017).
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34 477 However, we found no support for this third prediction. We did not find higher $\delta^{15}\text{N}$
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36 478 values in the three arthropods collected at the Lékédi Park than what we found in a
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38 479 variety of plant foods (Table S1). Further, females did not show higher average $\delta^{15}\text{N}$
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40 480 values than males across age classes. In fact, we found the opposite: females generally
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42 481 showed slightly lower $\delta^{15}\text{N}$ values than males, which we think is mainly driven by the
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44 482 higher $\delta^{15}\text{N}$ levels in productively active and thus nutritionally stressed male mandrills.

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49 483 This is the first isotopic investigation conducted in a large population of free-ranging
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51 484 mandrills. Our results partly confirm observational data, but also highlighted some
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53 485 potential future avenues for behavioral research, particularly regarding dietary
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55 486 differences between age classes as well as nutritional stress in reproductively active
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58 487 males.
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6

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689 **Table 1:** Results of the four Mixed Models on the responses of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in hair
 690 sections and blood samples, with the fixed effects of the season, individual age and sex.

Response	Sample sizes	Fixed effects	Statistics	p-value
$\delta^{13}\text{C}_{\text{hair}}$	214 hair sections	Season	F=3.82	0.024
	62 hair samples	Age	F=13.22	<0.001
	37 individuals	Sex	F=5.00	0.027
		Sex*Season	F=3.33	0.039
		Age*Season	F=3.06	0.050
$\delta^{13}\text{C}_{\text{blood}}$	46 blood samples	Season	F=21.52	<0.001
	36 individuals	Age	F=8.09	0.007
		Sex	F=0.73	0.400
$\delta^{15}\text{N}_{\text{hair}}$	208 hair sections	Season	F=0.93	0.400
	61 hair samples	Age	F=2.23	0.140
	37 individuals	Sex	F=4.34	0.004
		Sex*Age	F=6.51	0.012
$\delta^{15}\text{N}_{\text{blood}}$	46 blood samples	Season	F=33.55	<0.001
	36 individuals	Age	F=7	<0.001
		Sex	F=8.46	0.006
		Sex*Age	F=24.58	<0.001

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4 692 **Figure captions**
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10 694 **Figure 1:** Whisker boxplots with median, upper/lower quartiles and min/max ranges
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12 695 illustrating the effect of seasonal differences in mandrill isotope values between the long
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14 696 dry and the long rainy seasons revealed by the $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values of mandrill
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17 697 blood.
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23 699 **Figure 2:** Whisker boxplots with median, upper/lower quartiles and min/max ranges
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25 700 illustrating the effect of seasonal differences in mandrill $\delta^{13}\text{C}$ values of hair by sex (A:
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27 701 males, B: females) and age class (young <10 years, old > 10 years). Two age classes
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29 702 were defined here for the sake of clarity, although age was considered as a continuous
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31 703 predictor in our statistical models.
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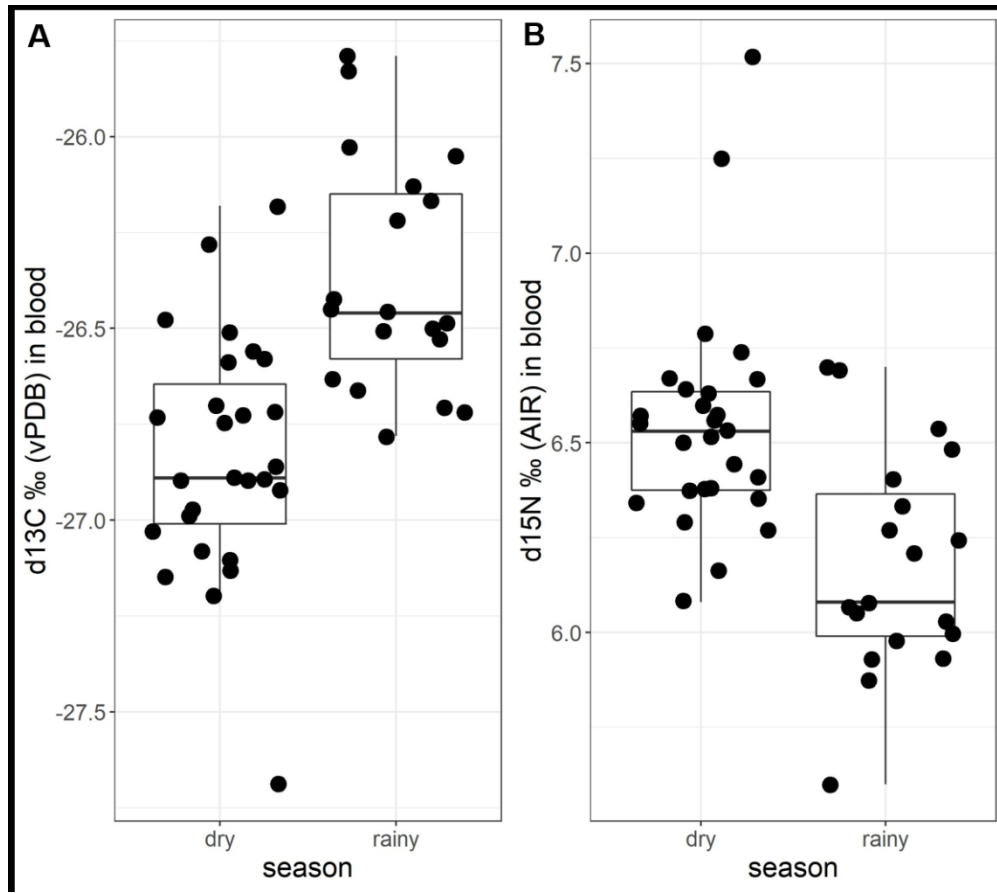
38 705 **Figure 3:** Scatter plot showing the relationship between individual age and $\delta^{13}\text{C}$ values
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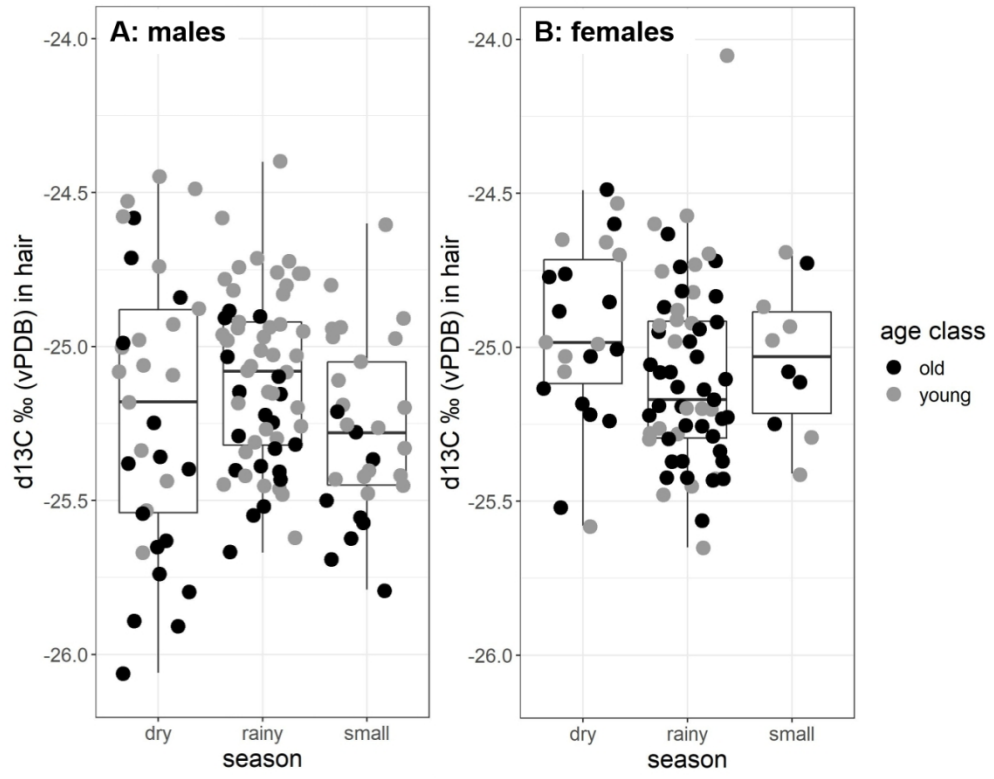
46 708 **Figure 4:** Scatter plots showing the relationships between individual age in years and
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48 709 $\delta^{15}\text{N}$ values in mandrill hair (grey symbols) and blood (black symbols) separate for
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50 710 males (A) and females (B).
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Research Highlights

- Stable isotope analyses of hair and blood have the potential to provide short and long-term data on how individual diets may shift over the season and with individual traits.
- Dietary patterns differ across seasons, between sexes as well as between young and older mandrills.
- Mature and reproductively active male mandrills showed the highest nitrogen isotope values as a result of nutritional stress possibly associated to intense male-male competition in this species.
- This is the first study showing isotopic evidence for nutritional stress in a free-ranging primate species.

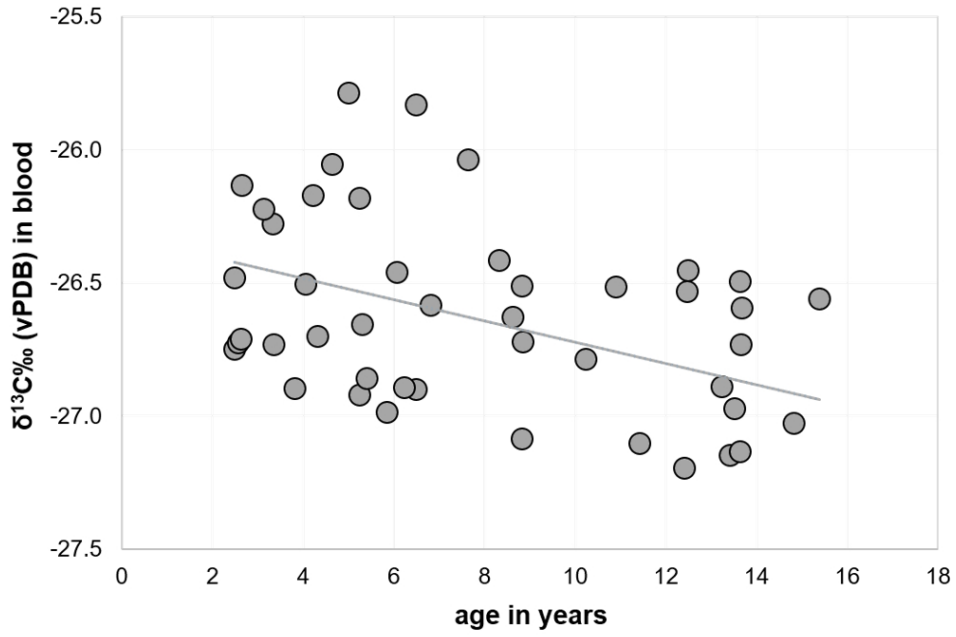


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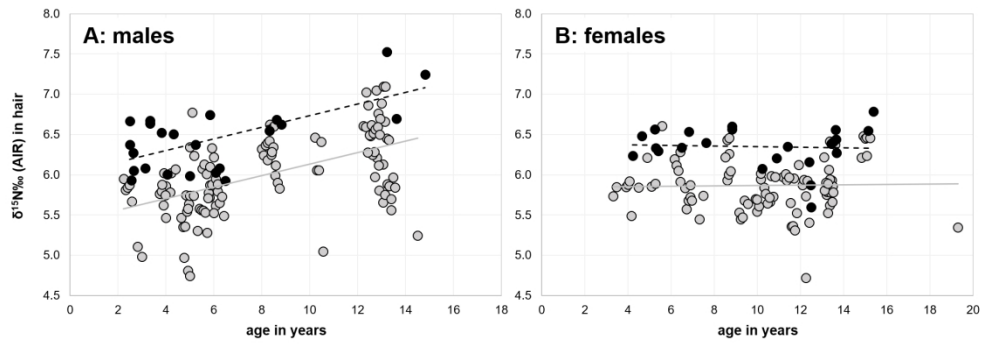


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Supplementary information:

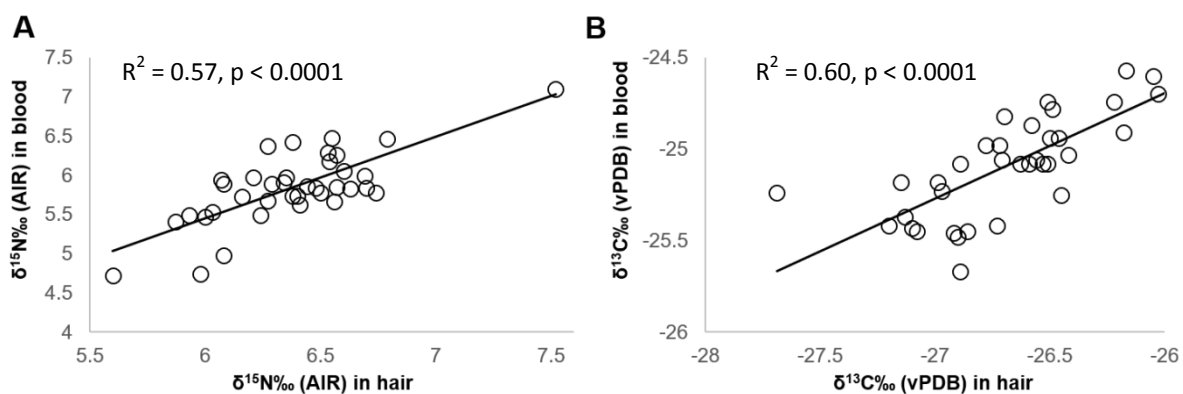
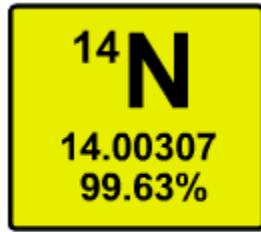


Figure S1: The $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) values measured in hair sections and blood samples, each obtained from the same individuals calculated to be representing overlapping time windows (n=37 samples). Blood and hair isotope values are significantly correlated.

Table S1: Stable isotopic values of a small selection of mandrill food items from Lékédi park. Insects and mushrooms were not determined to species level.

Species	Food category	Plant item	$\delta^{15}\text{N}\text{‰}$ AIR	$\delta^{13}\text{C}\text{‰}$ VPDB
Ants (unidentified)	fauna	-	5.3	-25.2
Butterfly (unidentified)	fauna	-	1.5	-27.9
Caterpillar (unidentified)	fauna	-	6.7	-26.4
Mushroom 1 (unidentified)	na	-	9.0	-29.1
Mushroom 2 (unidentified)	na	-	3.0	-32.1
Mushroom 3 (unidentified)	na	-	6.8	-24.0
<i>Megaphrynium macrostachum</i>	na	leaves/fruits	4.4	-31.5
<i>Smilax anceps</i>	na	stems/seeds	2.9	-29.2
<i>Aframomum cf polyanthum</i>	na	unknown	2.3	-32.8
<i>Croton sylvaticus</i>	na	unknown	5.1	-29.7
<i>Geophila afzelii</i>	na	unknown	1.5	-34.7
<i>Renealmia macrocolea</i>	na	unknown	3.6	-33.1
<i>Uapaca guineensis</i>	na	unknown	4.0	-29.0
<i>Dialium dinklagei</i>	non-reproductive part	leaves	6.4	-32.1
<i>Lasianthus batangensis</i>	non-reproductive part	leaves	3.4	-35.0
<i>Megaphrynium macrostachum</i>	non-reproductive part	leaves	11.6	-29.9
<i>Nymphaea maculata</i>	non-reproductive part	leaves	6.6	-28.7
<i>Psychotria stenostegia</i>	non-reproductive part	stems/seeds	1.8	-35.4
<i>Cnestis corniculata</i>	reproductive part	flowers	5.4	-31.6
<i>Psychotria gillettii</i>	reproductive part	flowers	3.8	-28.9
<i>Psychotria stenostegia</i>	reproductive part	fruits	3.0	-34.0
<i>Cnestis corniculata</i>	reproductive part	seeds	2.9	-31.9
<i>Hylodendron gabunense</i>	reproductive part	seeds	4.4	-26.7
<i>Psychotria gillettii</i>	reproductive part	seeds	2.5	-28.9



Stable



Stable



Photo-credit: Paul Amblard-Rambert

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