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NEWS AND PERSPECTIVES

Measures of food intake in mantled howling monkeys

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Abstract Food intake (i.e., the amount of food consumed by an individual) is a crucial measure for studying feeding behavior, but its measurement requires high visibility of individuals and long recording sessions, which are often difficult to accomplish under field conditions. As a consequence, studies on the feeding behavior of primates typically do not estimate food intake directly, and focus rather on studying dietary patterns through indirect measures of food intake, such as time spent feeding, number of food bites and food intake rates. The aim of the present study was to determine the validity of these estimators of food intake in mantled howling monkeys (*Alouatta palliata*) by comparing the estimations with the direct measurement of food intake. We recorded 97 feeding episodes of two male and two female adults, during which we determined the number of ingested food units (i.e., number of leaves and number of fruits), the number of bites taken and time spent feeding. After weighing units of food similar to those consumed, we calculated food intake and mean intake rates per food type (ripe fruits, unripe fruits, mature leaves, and young leaves). The number of bites taken by mantled howling monkeys during feeding episodes was strongly related to food intake, and this relationship was not affected by the type of food ingested. In contrast, neither time spent feeding nor food ingestion rate were related to food intake. These results suggest that the number of bites could be used as a valid proxy to study food intake in this species, whereas the other two measures are likely to yield inaccurate estimates of food intake.

Keywords *Alouatta* · Bite counts · Feeding · Foraging · Los Tuxtlas · Time spent feeding

Introduction

Food intake, a crucial measure for estimating nutrient acquisition through direct observation, is defined as the amount of food in grams consumed by an individual (Zinner 1999; Rothman et al. 2013), and feeding rates, which describe food intake per min (i.e., g/min: Nakagawa 2009), have been used to study the feeding ecology of many primate species (reviewed in Nakagawa 2009). However, in order to obtain reliable estimates of food intake, high visibility of individuals and long recording sessions are required, both of which are difficult under field conditions, especially in the study of arboreal primates. As a consequence, the majority of studies on primate feeding behavior do not estimate food intake, and focus rather on quantifying the time spent feeding on different foods.

Variation in physical and chemical properties among food types leads to differences in processing effort (e.g., chewing: Wright et al. 2008; Norconk et al. 2009), which are then reflected in differences in processing time. As a consequence, time spent feeding may not be a reliable measure of the dietary patterns of primate species that consume food types with marked differences in physical or chemical properties, as first noted by Hladik (1977). For instance, in a comparison of the feeding rates for fruits and leaves among six primate species from seven populations,

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the feeding rates for fruits were significantly higher than those for leaves in two of the seven populations, whereas no significant differences were found among the remaining five populations (Nakagawa 2009). These findings suggest that feeding time occasionally results in underestimation, but seldom overestimation, of fruit intake by weight (Nakagawa 2009). Additionally, the rate of food intake varies within feeding bouts (Chivers 1974), both within and between individuals (Zinner 1999). The shortcomings of the use of time spent feeding as a reliable measure of primate food intake, while frequently mentioned (e.g., Rothman et al. 2012, 2013), have not often been quantified (but see Hladik 1977; Gaulin and Gaulin 1982; Kurland and Gaulin 1987; Nakagawa 1997; Chivers 1998; Zinner 1999). An additional measure used as a proxy for food intake is the frequency with which individuals insert food into their mouths, usually referred to as bites (Schülke et al. 2006; Sayers et al. 2010; Rothman et al. 2012). Because it is part of the feeding process, bite number is potentially a more valid measure of food intake than time spent feeding.

The dietary patterns of howling monkeys (*Alouatta* spp.) have been thoroughly studied over the last 80 years, with a recent review identifying 163 studies on the diets of these primates (Dias and Rangel-Negrín 2015). Among these, 159 were based on direct observation of individuals, and 142 provided data on the dietary patterns of howling monkeys based on the time spent feeding. In a study of red howler monkeys (*A. seniculus*), Gaulin and Gaulin (1982) found that the time spent feeding underestimated fruit consumption and overestimated leaf consumption compared to estimations of dry weight consumed per food type. These results support contentions that the use of time spent feeding is not a reliable method for studying feeding behavior, and question the accuracy of time-based classifications of dietary patterns of howling monkeys (Garber et al. 2015). It is important, therefore, to assess the validity of using measures of time spent consuming different food types for determining dietary patterns. Moreover, given the species' arboreal lifestyle, which hinders detailed observation of feeding behavior, finding alternative measures for the study of food intake by howling monkeys is imperative.

In the present study, we focused on the feeding behavior of *A. palliata*, the howling monkey species for which more research on dietary patterns has been conducted (Dias and Rangel-Negrín 2015). Our aim was to determine the validity of three estimators of food intake via direct observation: the number of bites, the time spent feeding and mean intake rates. The last of these was calculated by multiplying time spent feeding per food item by the average rate of food intake per food type (i.e., fruits, leaves, etc.) reported in the literature (Dias et al. 2014; Garber et al. 2015).

Methods

Study site and subjects

The study was conducted at La Flor de Catemaco, Veracruz, Mexico (18°26′39″ N, 95°02′57 W), a 124-ha ranch dedicated to the commercial production of ornamental plants, mainly parlour palms (*Chamaedorea elegans*). Although the original understory and forest floor vegetation were replaced by the palm plantations, the canopy and emergent strata (corresponding to tropical evergreen forest) at this site are preserved, because palms are grown in the shade of trees.

A total of 25 mantled howling monkeys, divided into three groups, live in this area. This population has been studied since 2004 (Shedden-González and Rodríguez-Luna 2010), and daily observations have been conducted continuously since 2012. Individuals are easily identified by their natural anatomical and physiognomic characteristics, including body size and proportions, scars, broken fingers, genital morphology and pigmentation, as well as blond hairs and skin pigmentation on the feet, hands and tail. Additionally, several individuals are marked with ankle bracelets. We focused on one group comprising two adult males, two adult females, one juvenile and one infant, and observed the feeding behavior of the adults.

Observations of feeding behavior

From April to May 2013, we opportunistically recorded the feeding behavior of the study subjects. Feeding was defined as the ingestion, chewing and swallowing of food. When visibility conditions allowed for the observation of feeding without interference from surrounding vegetation, we began focal animal sampling combined with continuous recording when a subject was observed feeding (Altmann 1974). Focal samples were interrupted when visibility was lost. Two focal samples of the same individual were separated by at least 5 min. During each focal sample (hereafter, feeding episode), we collected the following data: start and end time of feeding; food types consumed, classified as ripe fruit, unripe fruit, mature leaves or young leaves (individuals did not consume other plant parts during the study); number of units (a unit was one fruit or one leaf) of the food item consumed; number of bites; and plant species. A bite was defined as each occasion in which food was put into the mouth during a feeding episode. When subjects did not ingest a unit with a single bite, we noted the proportion of the unit put into the mouth (e.g., half, quarter, etc.). We also noted other characteristics of foods that assisted in the collection of items similar to those ingested by subjects (explained below), such as color or position within the tree crown.

Over the course of ca. 300 observation-hours, we sampled a total of 97 feeding episodes, of which 25 corresponded to the ingestion of ripe fruits, 24 to unripe fruits, 24 to mature leaves, and 24 to young leaves. The duration of feeding episodes varied between 3 and 69 s (mean ± SD = 26.1 ± 14.5 s). Note that the total number of feeding episodes analyzed here corresponds to those in which all information described above could be recorded, not to the total number of feeding episodes observed during the 300 h.

Food intake estimations

Following observations of feeding episodes and after the monkeys had moved to a different tree, we used a tree pruner to collect ten food units similar to those ingested by the subject for each food type in each tree that was used as a food source. These units were collected directly from trees at the approximate location within the tree crown where the subjects consumed comparable units. Food samples were preserved in plastic bags inside a cooler while in the field, and weighted in fresh at the end of the day in the field station. Each unit was weighed to the nearest milligram using a digital scale (VB-240; VELAB Microscopes Inc., IL, USA). Based on these measurements, we calculated mean unit weight per food type per tree (Table 1). Food intake was calculated as the sum of the weight of all units ingested during each feeding episode. Subjects ingested a single food type in all recorded feeding episodes.

Data analysis

To estimate mean intake rate per food type, following previous descriptions of this measure (Amato and Garber 2014; Garber et al. 2015), we calculated mean food intake per unit of time (seconds in our study) per food item (Table 1) and averaged this rate per food type (i.e., ripe fruit, unripe fruit, mature leaf or young leaf). These average rates were then multiplied by time spent feeding per feeding episode according to the food type that was ingested.

We used generalized linear mixed models (GLMMs; Rabe-Hesketh et al. 2005) to determine whether food intake could be predicted by the following factors: (1) time spent feeding, (2) number of bites, and (3) mean intake rate. In each model we also included the interaction between each factor and the type of food consumed to determine whether the relationship between food intake and each food intake proxy was affected by food type. We checked that the assumptions of normally distributed and homogeneous residuals were fulfilled through visual inspection of QQ plots. Individual identity was used as a random factor in all models. All analyses were performed with SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). The statistical threshold was set at $P < 0.05$.

Results

The number of bites was a significant predictor of food intake ($R^2 = 0.85$, $F_{1,92} = 116.5$, $P < 0.001$; Fig. 1a), and this relationship did not vary by food type that was consumed ($F_{3,40} = 2.6$, $P = 0.067$). In contrast, neither time spent feeding ($F_{1,92} = 0.01$, $P = 0.998$) nor mean intake rate ($F_{1,92} = 0.09$, $P = 0.682$) were related to food intake. Food intake was predicted, however, by the interaction between time spent feeding and food type ($F_{3,40} = 8.1$, $P = 0.025$, Fig. 1b). Specifically, the predictive value of

Table 1 Mean (± SD) weight (g) of food units per food type among 10 plant species consumed by mantled howling monkeys and mean rates of intake (g/s) per food type

Species[a]	Ripe fruits	Unripe fruits	Mature leaves	Young leaves
Albizia purpusii (2)	0.815 (0.03)	–[b]	–	0.135 (0.288)
Brosimum alicastrum (3)	2.279 (2.207)	–	–	–
Bursera simaruba (2)	0.204 (0.126)	–	–	0.046 (0.159)
Ficus americana (1)	0.499 (0.014)	–	–	0.047 (0.089)
Ficus apollinaris (1)	0.172 (0.025)	–	0.172 (0.147)	0.187 (0.179)
Ficus aurea (11)	0.696 (0.475)	0.733 (0.406)	0.519 (0.804)	0.447 (0.452)
Ficus ovalis (6)	0.153 (0.027)	0.143 (0.041)	0.182 (0.189)	0.176 (0.163)
Ficus yoponensis (4)	0.946 (0.327)	0.953 (1.189)	–	0.949 (0.879)
Spondias mombin (1)	–	–	–	0.213 (0.159)
Spondias radlkoferi (1)	0.236 (0.169)	–	–	0.103 (0.045)
Mean intake rate (g/s)	0.206	0.118	0.077	0.076

[a] Numbers in parentheses represent the number of trees from each plant species that were used as food sources by howling monkeys. When food units were collected from more than one tree, we divided the sum of the mean unit weights per tree by the number of sampled trees

[b] Food type not ingested from this plant species

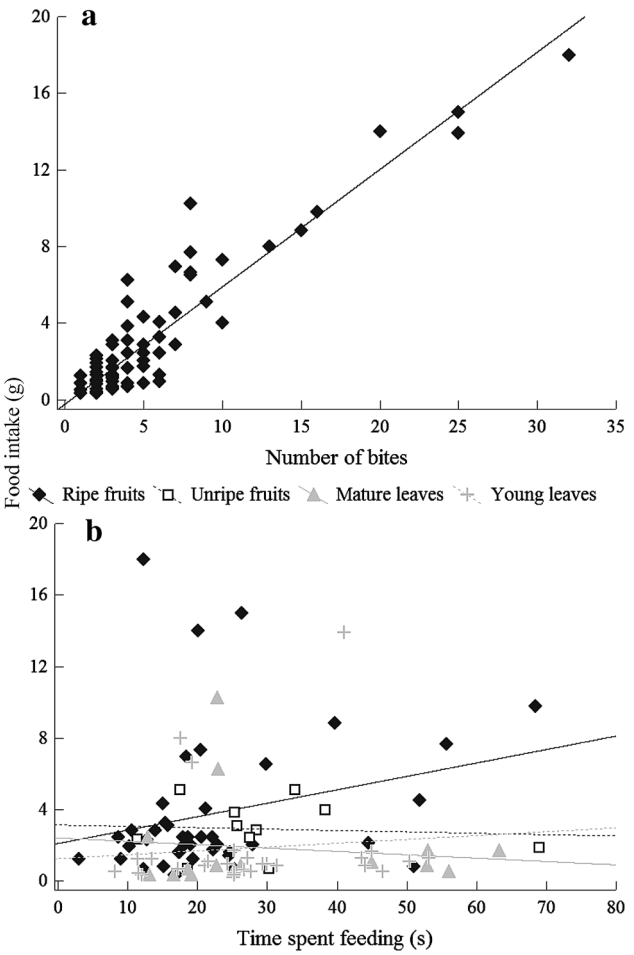

Fig. 1 Relationship between food intake (g) and both **a** the number of bites and **b** time spent feeding according to the food type consumed in feeding episodes ($N = 97$) of mantled howling monkeys

the relationship between time spent feeding and food intake was stronger for ripe fruits ($R^2_{25} = 0.07$, $P = 0.096$) than for unripe fruits ($R^2_{24} = 0.01$, $P = 0.823$), mature leaves ($R^2_{24} = 0.01$, $P = 0.661$), and young leaves ($R^2_{24} = 0.01$, $P = 0.646$). The interaction between mean intake rate and food type was not predictive of food intake ($F_{3,40} = 1.4$, $P = 0.512$).

Discussion

First, we must note that our data set represents snapshots of the feeding behavior of mantled howling monkeys in this population, as our study was short-term in nature, and we recorded the behavior of only two males and two females. Furthermore, although we worked in a forest where the canopy reaches 45 m in height, study subjects were well habituated to our presence, which facilitated our observations. With this in mind, our results indicate that the number of bites taken by mantled howling monkeys during feeding episodes is strongly related to food intake, and this relationship is not affected by the food type ingested. In contrast, neither time spent feeding nor mean intake rate are related to food intake. These results suggest that the number of bites may be used as a valid proxy for the study of food intake in this species, whereas the other two measures are likely to yield incorrect estimates of food intake.

Bite counting has been used to assess food intake in numerous studies of primates (e.g., red howling monkeys, *A. seniculus*: Oftedal 1992; red-tailed monkeys, *Cercopithecus ascanius*: Rode et al. 2006) and other mammals (e.g., cows, *Bos primigenius taurus*: Vance et al. 2012; goats, *Capra hircus*: Egea et al. 2014). Although individuals sometimes reject food after biting (e.g., Arroyo-Rodríguez et al. 2015), when a bite is taken, it usually leads to the swallowing of food, resulting in a positive relationship between bite counts and food intake. It is noteworthy that although foods consumed by subjects in our study varied in weight (e.g., *Brosimum alicastrum* ripe fruits vs. *Ficus americana* young leaves), the relationship between the number of bites and food intake was not affected by the type of food. This result is probably linked to the variation among food types in the mean number of food units consumed per feeding episode (mature leaves = 7.5; young leaves = 8.6; ripe fruits = 6.3; unripe fruits = 6.3), along with the variation in unit weight per food type (Table 1, Milton 1984), such that more units of the lighter foods were consumed per bite than units of heavier types.

In contrast to bite counts, time spent feeding and mean intake rates were not good predictors of food intake. Several previous studies have noted that, because it is dependent on processing requirements, time spent feeding is a poor proxy for food intake (Hladik 1977; Gaulin and Gaulin 1982; Zinner 1999; Nakagawa 2009). Food toughness, for instance, affects chewing, and is not always positively correlated with consumed mass (Rothman et al. 2012), thus biasing time estimates in favor of hard foods. The significant interaction that we found between time spent feeding and food type in predicting food intake is consistent with this evidence. Mean intake rate, aside from incorporating time spent feeding (with the shortcomings already discussed), is a measure that likely overlooks important variation within food types in weight, size, and chemical and physical properties, as it is based on average weight per food type (e.g., Amato and Garber 2014). In our sample of food types, for instance, there was a greater than tenfold difference between the minimum and maximum weight of ripe fruits and young leaves. Our results, therefore, agree with previous contentions that time-based measures of feeding behavior are not appropriate for the study of food intake in primates (Kurland and Gaulin 1987;

Zinner 1999; Nakagawa 2009; Rothman et al. 2012), and specifically in howler monkeys (Gaulin and Gaulin 1982; Chivers 1998). It is also important to note that analysis of C-peptides, ketone bodies and stable isotopes currently enables the study of the nutritional status of primates without direct observation (e.g., Rothman et al. 2013). The use of these methods should increase our knowledge of the nutritional ecology of primates for which detailed observations are not possible.

In the present study, we were able to analyze only a small number of feeding episodes, as we were required in each episode to record the number and characteristics of consumed food units, the number of bites taken and time spent feeding, and this was not always possible, due to poor visibility of focal animals. In our experience, however, recording bite counts per feeding episode was much easier than counting consumed food units, so we believe that the use of this measure is feasible for studying the feeding behavior of mantled howling monkeys. With regard to the abundant data on time spent feeding by howling monkeys (Dias and Rangel-Negrín 2015), if our results are confirmed by future testing under diverse conditions (e.g., diets including more food types), the data on time spent feeding should no longer to used to define dietary habits (e.g., "howlers consume significantly more leaves than fruits": Dias and Rangel-Negrín 2015: p 41) or nutrient selection [e.g., "…the seasonal foods howlers preferred (measured as time spent consuming them) were generally of a relatively high nutritional quality": Milton 1980: p 98]. In light of the shortcomings of time spent feeding as a measure of feeding behavior, it is possible that much less is known about the feeding behavior of howling monkeys—and much more about foraging efforts—than was previously assumed.

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References

Altmann J (1974) Observational study of behavior: sampling methods. Behaviour 49:227–267

Amato K, Garber PA (2014) Nutrition and foraging strategies of the black howler monkey (*Alouatta pigra*) in Palenque National Park, Mexico. Am J Primatol 76:774–787

Arroyo-Rodríguez V, Andresen E, Bravo SP, Stevenson PR (2015) Seed dispersal by howler monkeys: current knowledge, conservation implications, and future directions. In: Kowalewski M, Garber PA, Cortés-Ortiz L, Urbani B, Youlatos D (eds) Howler monkeys: behavior, ecology, and conservation. Springer, New York, pp 111–139

Chivers DJ (1974) The siamang in Malaya: a field study of a primate in tropical rain forest. Contr Primatol 4:1–335

Chivers DJ (1998) Measuring food intake in wild animals: primates. Proc Nutr Soc 57:321–332

Dias PAD, Rangel-Negrín A (2015) Diets of howler monkeys. In: Kowalewski M, Garber PA, Cortés-Ortiz L, Urbani B, Youlatos D (eds) Howler monkeys: behavior, ecology, and conservation. Springer, New York, pp 21–56

Dias PAD, Rangel-Negrín A, Coyohua-Fuentes A, Canales-Espinosa D (2014) The dietary breadth of a Neotropical primate is not associated with vegetation disturbance. Am J Primatol 76:1151–1162

Egea AV, Allegrettia L, Paez Lama S, Grilli D, Sartor C, Fucili M, Guevara JC, Passera C (2014) Selective behavior of Creole goats in response to the functional heterogeneity of native forage species in the central Monte desert, Argentina. Small Rumin Res 120:90–99

Garber PA, Righini N, Kowalewski MM (2015) Evidence of alternative dietary syndromes and nutritional goals in the genus *Alouatta*. In: Kowalewski M, Garber PA, Cortés-Ortiz L, Urbani B, Youlatos D (eds) Howler monkeys: behavior, ecology, and conservation. Springer, New York, pp 85–109

Gaulin SJC, Gaulin CK (1982) Behavioral ecology of *Alouatta seniculus* in an Andean cloud forest. Int J Primatol 3:1–32

Hladik CM (1977) A comparative study of the feeding strategies of two sympatric leaf monkeys: *Presbytis senex* and *Presbytis entellus*. In: Clutton-Brock TH (ed) Primate ecology. Academic Press, New York, pp 324–353

Kurland JA, Gaulin SJC (1987) Comparability among measures of primate diets. Primates 28:71–77

Milton K (1980) The foraging strategy of howler monkeys: a study in primate economics. Columbia University Press, New York

Milton K (1984) The role of food processing factors in primate choice. In: Rodman P, Cant J (eds) Adaptations for foraging in nonhuman primates. Columbia University Press, New York, pp 249–279

Nakagawa N (1997) Determinants of the dramatic seasonal changes in the intake of energy and protein by Japanese monkeys in a cool temperate forest. Am J Primatol 41:267–288

Nakagawa N (2009) Feeding rate as valuable information in primate feeding ecology. Primates 50:131–141

Norconk MA, Wright BW, Conklin-Brittain NL, Vinyard CJ (2009) Mechanical and nutritional properties of food as factors in platyrrhine dietary adaptations. In: Garber PA, Bicca-Marques JC, Estrada AE, Heymann EW, Strier KB (eds) Mechanical and nutritional properties of food as factors in platyrrhine dietary adaptations. Springer, New York, pp 279–319

Oftedal OT (1992) The nutritional consequences of foraging in primates: the relationship of nutrient intakes to nutrient requirements. Philos T Roy Soc B 334:161–170

Rabe-Hesketh S, Skrondal A, Pickles A (2005) Maximum likelihood estimation of limited and discrete dependent variable models with nested random effects. J Econom 128:301–323

Rode KD, Chapman CA, McDowell LR, Stickler C (2006) The role of nutrition in population: a comparison of redtail monkeys diets and densities across habitats and logging intensities. Biotropica 38:625–634

Rothman JM, Chapman CA, van Soest PJ (2012) Methods in primate nutritional ecology: a user's guide. Int J Primatol 33:542–566

Rothman JM, Vogel ER, Blumenthal SA (2013) Diet and nutrition. In: Blair M, Bynum N, Sterling E (eds) Primate ecology and conservation: a handbook of techniques. Oxford University Press, Oxford, pp 195–212

Sayers K, Norconk MA, Conklin-Brittain NL (2010) Optimal foraging on the roof of the world: Himalayan langurs and the classical prey model. Am J Phys Anthropol 141:337–357

Schülke O, Chalise MK, Koening A (2006) The importance of ingestion rates for estimating food quality and energy intake. Am J Primatol 69:951–965

Shedden-González A, Rodríguez-Luna E (2010) Responses of a translocated howler monkey *Alouatta palliata* group to new environmental conditions. Endang Species Res 12:25–30

Vance ER, Ferris CP, Elliott CT, Kilpatrick DJ (2012) A comparison of the feeding and grazing behaviour of primiparous Holstein-Friesian and Jersey×Holstein-Friesian dairy cows. Irish J Agr Food Res 51:45–61

Wright BW, Ulibarri L, O'Brien J, Sadler B, Prodhan R, Covert HH, Nadler T (2008) It's tough out there: variations in toughness of ingested leaves and feeding behavior among four Colobinae in Vietnam. Int J Primatol 29:1455–1466

Zinner D (1999) Relationship between food intake in hamadryas baboons (*Papio hamadryas*) and the value of feeding time as predictor of food intake. Zoo Biol 18:495–505