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FAECAL ANALYSIS AS A COMPLEMENTARY APPROACH TO THE STUDY OF FEEDING ECOLOGY IN THE COMMON MARMOSET (*Callithrix jacchus* LINNAEUS, 1758)

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ABSTRACT. We evaluated the application of faecal analysis as a complementary approach to direct observation in the study of *Callithrix jacchus* feeding ecology. Our results indicate that animal foods may be more commonly consumed (based on faecal samples-FS; 61.48% of diet) than previously thought (e.g., only 22.83% based on observational data-OD). Whereas FS provide better data on animal prey, it was less effective than OD in identifying plant items. Six insect orders—including Dermaptera and two hymenopteran families, not previously recorded in marmoset diet—were identified through FS, which is thus shown to be useful in characterizing diet, especially in insectivorous primates.

RESUMO. Análise fecal como uma abordagem complementar no estudo da ecologia alimentar do sagui comum (*Callithrix jacchus* Linnaeus, 1758). Nós avaliamos a aplicação da análise fecal como método complementar à observação direta no estudo da Ecologia alimentar de *Callithrix jacchus*. Nossos resultados indicam que alimentos de origem animal podem ser consumidos com uma frequência maior (com base na análise fecal-FS; 61.48% da dieta) do que previamente pensado (e.g., apenas 22.83% com base na observação direta-OD). Apesar de fornecer melhores dados sobre presas, FS foi menos eficiente que a observação direta no reconhecimento de itens vegetais. Seis Ordens de insetos —incluindo Dermaptera e duas Famílias de Hymenoptera, nunca antes registradas na dieta de saguis— foram identificadas através da FS, que, portanto, se mostrou útil na caracterização da dieta, especialmente para primatas insetívoros.

Key words: Arthropods. Diet. Marmosets. Methods.

Palavras chave: Artrópodes. Dieta. Métodos. Sagui.

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While providing useful data on primate ecology, behavioural studies are susceptible to observational bias, mostly related to differential visibility of specific behaviours and intrinsic limitations to observers or those imposed by field conditions (Ferrari and Rylands, 1994). On this matter, the collection of prey feeding data of small-bodied arboreal monkeys such as marmosets and tamarins (Callitrichidae), may be especially vulnerable to those bias (Ferrari and Rylands, 1994). Behavioural data collection is the main method used to evaluate the ecological traits of primate species, in a general manner, and it is especially important for early descriptions of the autoecology of any given species (e.g., Alonso and Langguth, 1989; Passamani, 1998; Silva et al., 2011, among others).

At most sites, the common marmoset (*Callithrix jacchus*) feeds primarily on plant exudates, whereas animal prey generally contributes less than 10% of feeding records, although other marmosets may be more insectivorous (Martins and Setz, 2000), and one study based on the analysis of stomach contents in golden-handed tamarins (*Saguinus midas*) indicated that behavioural data may underestimate the consumption of animal prey considerably (Pack et al., 1999). Thus, alternative or complementary methods to behavioural sampling have been discussed in a number of studies (e.g., Moreno-Black, 1978; Su and Lee, 2001; Bradley et al., 2007; Pickett et al., 2012), and all have emphasized the need for the more systematic collection of complementary data.

Here, the diet of a free-ranging group of common marmosets (*Callithrix jacchus*) was analysed using two methods—behavioural monitoring and the analysis of faecal samples. The study aimed to evaluate the importance and viability of faecal analysis as a complementary approach to data on feeding behaviour of *Callithrix jacchus* (mainly the behaviour of prey feeding) and, by extension, other insectivorous primate species. We also indicate how to process data and run specific analysis when dealing with the feeding ecology of primates, especially insectivorous ones.

Data were collected on the 15-hectare São Cristóvão campus of the Federal University of Sergipe (UFS) in Northeastern Brazil (10º 55' S, 37º 06' W). The *Callithrix jacchus* study group inhabits a home range of approximately 3.5 hectares, and contained four members during the study—an adult pair, a juvenile, and an infant. Behavioural data were collected in standardised scan samples (Ferrari and Rylands, 1994), with a one-minute scan at five-minute intervals throughout the daily activity period (approximately 05:15-17:15 h). During each scan, the activity of each visible animal was recorded, and when feeding, the item was identified, whenever possible.

During monitoring, whenever an animal was observed defecating, an attempt was made to collect the faeces. The samples were stored in 50 mL plastic pots with 70% ethanol until processing in a Petri dish under a Wild Heerbrugg M3Z stereoscopic microscope. All recognizable items were separated for recognition and taxon identification. The seeds were compared with those found in fruits consumed by the marmosets and of other plants found within the study area. Arthropod fragments were identified by comparison with specimens available in the UFS Entomology Laboratory.

As the relative volume of items in the faecal matter could not be estimated reliably, the results are presented as the number of faecal samples in which a certain item (e.g., a taxon) occurred, and its relative frequency, that is the number of occurrence divided by the total number of faecal samples.

As the behavioural records and the faecal samples provided different and alternative parameters (see below), an attempt was made to approximate the two datasets by estimating the number of feeding events observed either on the faecal samples (henceforth denoted feeding events on samples), and through the behavioural sampling (henceforth denoted observed feeding events), rather than the time (behavioural records) spent feeding on each item. For the behavioural data, the approach used was adapted from Clutton-Brock's (1977) proportional method for the analysis of scan sample records, which compensates for contrasts in the relative visibility of different behaviours. In this case, each scan sample in which the item was observed was counted as a single event, irrespective of the number of records collected. Two or more consecutive scans in which the same item was registered were also counted as a single event. The number of feeding events on samples was estimated by counting the number of samples containing a given taxon.

We monitored the study group continuously during 12 separate days in April and May 2011, with a total of 133 hours of monitoring, which rendered 509 scan sample records of feeding behaviour, from which 111 were from the adult male, 135 from the adult female, 10 from one of the adults, 119 from the juvenile, and 134 from the infant. Simultaneously, a total of 52 faecal samples were collected, from which 17 were from the adult male, 16 from the adult female, five from unidentified adults, nine from the juvenile, and five from the infant. Because of differences related to foraging skills, we used only the records and samples from adult individuals for the comparison of the diet of the marmosets described by behavioural data and faecal samples. After this, we had 256 behavioural records and 38 faecal samples to analyse (**Table 1**).

According to the behavioural records, the adult marmosets fed primarily on fruit (74.22%), followed by a much smaller proportion (12.50%) of arthropod prey (**Table 2**). Even assuming that all the unidentified records represent the consumption of prey items (as considered by Martins and Setz, 2000, and Hilário and Ferrari, 2010), prey would still have represented only 16.41% of the diet. In the case of the number of observed feeding events (**Table 2**), absolute values decreased in all cases, but disproportionately more in the case of plant items in comparison with animal prey. If unidentified items are assumed to represent prey, up to 29.92% of the observed feeding events observed may have involved the consumption of animal material, although this assumption may be unreliable, given the presence of other items, which were not identified through behavioural monitoring, in the faecal samples, such as flowers and even seeds.

Table 1

Behavioural records and faecal samples collected from each of the individuals of the monitored group and the relative data sampling of adult individuals in São Cristóvão, Sergipe, Brazil.

Individual	Behavioural Records (%)	Faecal Samples (%)
Adult male	111 (21.8%)	17 (32.7%)
Adult female	135 (26.5%)	16 (30.8%)
Unidentified adults	10 (2.0%)	5 (9.6%)
Juvenile female	119 (23.4%)	9 (17.3%)
Infant female	134 (26.3%)	5 (9.6%)
Total	509	52
Total for Adults	256 (50.3%)	38 (73.1%)

Table 2

Comparison of the composition of the diet of the adult individuals of a group of *Callithrix jacchus* evaluated both through behavioural monitoring and the analysis of faecal samples in São Cristóvão, Sergipe, Brazil.

Item	Number (%) of:			
	Behavioural		Faecal	
	Records	Events[1]	Samples	Events[2]
Fruit	190 (74.22)	71 (55.91)		42 (31.11)
Gum	13 (5.08)	11 (8.66)		-
Nectar	8 (3.13)	6 (4.72)		-
Arthropod	32 (12.50)	29 (22.83)		83 (61.48)
Vertebrate	3 (1.17)	1 (0.79)		-
Flower	-	-		10 (7.41)
Unidentified	10 (3.91)	9 (7.09)		-
Total	256 (100.00)	127 (100.00)		135 (100.00)

[1] The number of observed feeding events was estimated by considering multiple records of the ingestion of only one item in the same scan as one event. Consecutive scans of the ingestion of the same item were also considered as one single event.

[2] In the case of faecal samples, a feeding event was defined as the presence of a given taxon (e.g. Hymenoptera) in a sample.

A total of 38 faecal samples were collected from the adult individuals of the group, representing a mean of 3.17 ± 3.04 samples per day. The study subjects were observed defecating more frequently, but the small size of the faeces (generally no more than 2 cm in length and 1 cm in width) often hampered searches. It nevertheless seems reasonable to assume that the specimens constituted a random and thus representative sample of the faeces produced by the marmosets and, in turn, of the food items they ingested.

While not directly comparable with the behavioural data, the faecal analysis provided a distinct picture of the feeding behaviour of the subjects, in particular, that they were far more insectivorous (**Table 2**). All of the samples contained recognisable residues of at least one type of food item, with a mean of 3.5 ± 1.5 different items per sample, and a maximum of six. No more than one species of flower was found in any given faecal sample, but many samples contained two or more taxa of seed/fruit (n = 10) or insect (n = 39), with some samples containing five different types of insects. Considering each taxon found in a faecal sample as a separate feeding event, there is a major shift in the configuration of the data (**Table 2**), with insects now dominating. The faecal samples not only indicated that the ingestion of insects was at least as common as fruit, but the diversity of prey items in the faeces was considerably higher than that recorded in the behavioural data.

During behavioural monitoring, the marmosets were observed feeding on the nectar of two species, *Syzygium* cf. *jambos* (90.91% of nectar feeding records) and *Clitoria fairchildiana*. Gum feeding was relatively rare and most records (94.74%) involved cashew trees, *Anacardium occidentale*. At least ten species provided fruit, although in five cases, only one event was observed, and no seeds were found in the faeces. Three of the four plant species most exploited by the marmosets for their fruit could be identified in the faecal samples. The seeds of two species—*Azadirachta indica* and *Syzygium cumini*—are relatively large (> 2 cm), and were invariably spat out by the marmosets, explaining their absence from the faeces. Bananas (*Musa paradisiaca*) have no seeds, while the peduncle, rather than the fruit of the cashew (*A. occidentale*) was consumed, and the fruit/seed was discarded.

The seeds of four species were found in the faecal samples. These include the two species most exploited (*Inga laurina* and *Schinus terebinthifolius*) and two unidentified taxa, which were not recorded through the behavioural monitoring of the group. Evidence of the ingestion of the fruit of a fifth species—*Mangifera indica*—was derived from the presence of the characteristic fibres in the faeces. However, the contrasting estimates between faecal samples and feeding records regarding the relative contribution of each different taxon to the diet is a result of the discrepancies between the two sampling procedures, and should be viewed with caution. While the faecal samples are mostly related to frequency of consumption, behavioural records are an estimation of time spent feeding, which is more closely related to the volume of food ingested. Furthermore, intrinsic differences in ingestion patterns also play an important role determining the detectability of a given item in faecal samples, or the visibility of the ingestion of an item by an observer.

The most striking difference between the two approaches was that of insect prey (**Table 3**). Prey are notoriously difficult to identify under field conditions, and in the present study, this may have been reinforced by the relatively small size of most items. Whereas only two prey taxa were identified during monitoring, the analysis of the faecal samples confirmed the presence of six insect orders plus Arachnida. In the case of the Hymenoptera, three families (Formicidae, Mutilidae, and Vespidae) were found in the faeces. Adult wasps (Vespidae) and velvet ants (Mutilidae) have not been previously recorded in marmoset diets. They are unlikely prey for these small monkeys, given their painful stings, although they were each identified reliably in only one sample, so they may have been ingested accidentally during the consumption of other items. However, more than 60% of the samples contained hymenopteran remains, which indicates that this insect order was a major component of the diet of the study group.

Table 3

Arthropod prey identified during the behavioural monitoring of the *C. jacchus* group in São Cristóvão, Sergipe, Brazil, and the analysis of the faecal samples collected during the study period.

Taxon	Number (%) of:	
	Feeding events	Faecal samples
Hemiptera	2 (3.92)[1]	40 (76.92)
Coleoptera[2]	-	37 (71.15)
Hymenoptera[3]*	-	32 (61.54)
Lepidoptera	1 (1.96)	4 (7.69)
Dermaptera*	-	1 (1.92)
Orthoptera	-	1 (1.92)
Arachnida	-	1 (1.92)
Unidentified	48 (94.12)	-

[1] Cicadidae;
[2] Includes Scarabaeidae;
[3] Includes one record each of adult representatives of the families Formicidae, Mutilidae, and Vespidae;
* New Taxa recorded in marmoset diets (Vespidae, Mutilidae and Dermaptera), due to faecal sample analysis.

Another unusual prey was an earwig (Dermaptera), also not recorded previously for marmosets. While only one item was recorded, which might be consistent with accidental ingestion, dermapterans are nocturnal insects, which hide in crevices during the day (Powell 2009), suggesting active predation. Evidence of the predation of orthopterans—considered to be typical marmoset prey (Rylands and Mittermeier, 2013)—was found only in one faecal sample.

As Callitrichids may defecate 3-5 times per day (Garber, 1988; Oliveira and Ferrari, 2000), it is likely that only a small sample of the faecal material produced by the study subjects was obtained, what may account for not all components of the diet been present on the sampling analysis presented here. However, given that retrieving all the faeces produced would be nearly impossible under field conditions, while it can be assumed that a representative sample of the faeces was collected, the data provided a quite different perspective on the diet of the marmosets in comparison with the behavioural record.

There were a number of absolute differences. No evidence was found in the faecal samples of the consumption of gum, nectar or vertebrates, whereas flowers were found only in the faecal samples. Although, it seems likely that the absence of flowers from one dataset was at least partly related to sampling vagaries, given that these items were of minor importance. On the other hand, the lack of hard parts in gum and nectar makes their visual detection in faeces nearly impossible. Additionally, the only observed predation of a vertebrate—a nestling bird—was recorded at the end of the last day of observational data sampling, after which no more faecal samples were collected, explaining its absence from that dataset.

Similarly, the absence from the faeces of the seeds of some species—most notably *Syzygium cumini*—was due to their large size, whereas the two morphospecies identified in faecal samples which were not observed during behavioural monitoring was, in turn, probably a result of the combination of small size of the fruits and low frequency of consumption by the study subjects. Nevertheless, the faecal samples provided evidence of an additional two plant species, that is, 18.2% of the total recorded in the study (11 species).

The most striking differences were found in the insect prey, with the faecal samples providing a detailed inventory of prey items, in stark contrast with the behavioural record. The reliable identification of prey items is a problem common to all behavioural studies of marmosets (e.g., Martins and Setz, 2000; Hilário and Ferrari, 2010), although the proportion of items identified in the present study was exceptionally low. Despite providing a more systematic inventory of animal prey, faecal samples may still be biased due to the reduced probability of preserving soft body parts, typical of insect larvae (Dickman and Huang, 1988; Kunz and Whitaker Jr., 1983). This problem may be at least partly resolved by a recently-developed molecular approach (Pickett et al., 2012).

While the faecal samples provide a new perspective on marmoset prey—including novel items such as dermapterans and mutilids—it remains unclear to what extent the data are representative. In particular, it would be useful to know whether the taxa identified in the faecal samples are typical of the small prey items that are not normally identified in behavioural studies or simply reflect the types of prey available at the site during the study period.

As the faecal and behavioural samples provide distinct perspectives on the feeding ecology of *C. jacchus*, the present study has provoked more questions than it has provided answers, in particular with regard to the quantification of the diet. While ingestion rates can be estimated more effectively in frugivorous/folivorous primates (Amato and Garber, 2014), the cryptic nature of prey feeding in insectivorous ones like marmosets creates an imbalance in the behavioural record, which may undermine the reliability of estimates of the composition of the diet. In the present study, the analysis of faecal samples indicated that the ingestion of prey was far more frequent than suggested by the behavioural sample, as also shown by Pack et al. (1999). However, the differential consumption and/or digestion of animal and plant items may also result in specific biases in their representation in the faeces (Kunz and Whitaker Jr., 1983; Dickman and Huang, 1988; Kirkpatrick et al., 2001). This is why most studies have been restricted to the analysis of the frequency of items—as in the present case—rather than relative volume (Moreno-Black, 1978).

Overall, then, while the analysis of faecal samples provided a useful complementary approach to the understanding of the composition of the marmoset diet, and underscored the problems of sampling biases, more work will be required in order to guarantee the reliable and systematic analysis of diets. This may include the refinement of both behavioural (e.g., events vs. records) and faecal (e.g., application of molecular techniques) procedures.

Given that events of prey feeding are markedly shorter and usually represented by the consumption of only one prey, in contrast to feeding events involving plant items, it is expected that even if the frequency of prey ingestion is the same as the frequency of ingestion of plant items, the total amount of food ingested as well as the time spent feeding will most likely be greater for events involving plant items than those involving prey. Thus, we believe that the behavioural monitoring of groups, which usually measures time spent in feeding, is a better option for evaluating the relative importance of different item categories (e.g. fruits, gum, arthropods) in the diet of primates, including insectivorous species. However, if the frequencies of consumption are to be analysed, complementary approaches, such as the estimation of observed feeding events and the frequency of different items in faeces should be adopted. Furthermore, the data presented suggests that the main application of the faecal analysis is to access the composition of the diet of study subjects complementarily to the data collected through behavioural monitoring, especially for species that prey on insects.

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LITERATURE CITED

ALONSO C and A LANGGUTH. 1989. Ecologia e comportamento de *Callithrix jacchus* (Primates: Callitrichidae) numa ilha de floresta Atlântica. Revista Nordestina de Biologia 6:105-137.

AMATO KR and PA GARBER. 2014. Nutrition and foraging strategies of the black howler monkey (*Alouatta pigra*) in Palenque National Park, Mexico. American Journal of Primatology 76:774-787.

BRADLEY BJ, M STILLER, DM DORAN-SHEEHY, T HARRIS, CA CHAPMAN, L VIGILANT, and H POINAR. 2007. Plant DNA Sequences from feces: Potential means for assessing diets of wild primates. American Journal of Primatology 69:699-705.

CLUTTON-BROCK TH. 1977. Methodology and measurement. Pp. 557-584, in: Primate ecology: Studies of feeding and ranging behaviour in lemurs, monkeys and apes (TH Clutton-Brock, ed.). London: Academic Press.

DICKMAN CR and C HUANG. 1988. The reliability of fecal analysis as a method for determining the diet of insectivorous mammals. Journal of Mammalogy 69:108113.

FERRARI SF and AB RYLANDS. 1994. Activity budgets and differential visibility in field studies of three marmosets (*Callithrix* spp.). Folia Primatologica 63:78-83.

GARBER PA. 1988. Diet, foraging patterns, and resource defense in a mixed species troop of *Saguinus mystax* and *Saguinus fuscicollis* in Amazonian Peru. Behaviour 105:18-33.

HILÁRIO RR and SF FERRARI. 2010. Feeding ecology of a group of buffy-headed marmosets (*Callithrix flaviceps*): Fungi as a preferred resource. American Journal of Primatology 72:515-521.

KIRKPATRICK RC, RJ ZOU, ES DIERENFELD, and HW ZHOU. 2001. Digestion of selected foods by Yunnan snub-nosed monkey *Rhinopithecus bieti* (Colobinae). American Journal of Physical Anthropology 114:156-162.

KUNZ TH and JO WHITAKER Jr. 1983. An evaluation of fecal analysis for determining food habits of insectivorous bats. Canadian Journal of Zoology 61:1317-1321.

MARTINS MM and EZF SETZ. 2000. Diet of buffy tufted-eared marmosets (*Callithrix aurita*) in a forest fragment in southeastern Brazil. International Journal of Primatology 21467-476.

MORENO-BLACK G. 1978. The use of scat samples in primate diet analysis. Primates 19:215-221.

OLIVEIRA ACM and SF FERRARI. 2000. Seed dispersal by black-handed tamarins, *Saguinus midas niger* (Callitrichinae, Primates): Implications for the regeneration of degraded forest habitats in eastern Amazonia. Journal of Tropical Ecology 16:709-716.

PACK KS, O HENRY, and D SABATIER. 1999. The insectivorous-frugivorous diet of the golden-handed tamarin (*Saguinus midas midas*) in French Guiana. Folia Primatologica 70:1-7.

PASSAMANI M. 1998. Activity budget of Geoffroy's marmoset (*Callithrix geoffroyi*) in an Atlantic forest in southeastern Brazil. American Journal of Primatology 46:333-340.

PICKETT SB, CM BERGEY, and A DI FIORE. 2012. A metagenomic study of primate insect diet diversity. American Journal of Primatology 74:622-631.

POWELL JA. 2009. Dermaptera. Pp. 372-375, in: Encyclopedia of insects (VH Resh and RT Cardé, eds.). New York: Academic Press.

RYLANDS AB and RA MITTERMEIER. 2013. Family Callitrichidae (marmosets and tamarins). Pp. 262-346, in: Handbook of the Mammals of the World, Volume 3: Primates. (RA Mittermeier, AB Rylands, and DE Wilson, eds.). Lynx Edicions, Barcelona.

SILVA GMM, KCS VERÍSSIMO, and MAB OLIVEIRA. 2011. Orçamento das atividades diárias de dois grupos de *Callithrix jacchus* em área urbana. Revista de Etologia 10:57-63.

SU H, and L LEE. 2001. Food habits of Formosan rock macaques (*Macaca cyclopis*) in Jentse, Northeastern Taiwan, assessed by fecal analysis and behavioral observation. International Journal of Primatology 22:359-377.