**Title:**

# Faecal particle size in free-ranging proboscis monkeys, *Nasalis larvatus*: Variation between seasons

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## Abstract

Reducing the size of food particles is crucial for herbivores. Seasonal dietary changes are known to influence animals' chewing efficiency. Proboscis monkeys (*Nasalis larvatus*) are foregut fermenters, with a high chewing efficiency allowing them to achieve very fine faecal particles. In this study, we investigated how proboscis monkeys' chewing efficiency varies among wet and dry seasons, hypothesising differences possibly related to diet change. Faecal particle size analysis is an established approach to estimate chewing efficiency in mammalian herbivores. We analysed 113 proboscis monkey faecal samples collected in the Lower Kinabatangan Wildlife Sanctuary, between 2015 and 2017. By following standard sieve analysis protocols, we measured a mean particle size $MPS_{(0.025\text{-}8)}$ of 0.45 ± 0.14 mm, and confirmed a previous result that proboscis monkeys have a very low faecal MPS. This study highlights a seasonal influence on proboscis monkeys' chewing efficiency, with smaller MPS (better chewing efficiency) during the wet season. During that time of the year, individuals may potentially change their diet, as all faecal samples contained intact seeds. Whether the seasonal MPS difference in proboscis monkeys is smaller than in other colobines due to their 'rumination' strategy remains to be investigated.

## Keywords

*Nasalis larvatus*, chewing efficiency, nutritional ecology, forestomach, diet change, foregut fermenter

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## Introduction

Reducing the size of food particles is crucial in herbivores that rely on gut microbiota to digest plant components. In herbivores, fibre digestion relies on digesta retention and particle size. To accomplish the same digestibility, large digesta particles will need longer retention times than smaller ones [Bjorndal et al., 1990]. Measuring faecal particle size by wet sieving analysis, is an established non-invasive approach to determine the chewing efficiency of mammals [Fritz et al., 2009]. Several studies focused on faecal particle size in mammals [Fritz et al., 2009; Clauss et al., 2015], or more specifically in ruminants [Renecker and Hudson, 1990; Clauss et al., 2002] and primates [Dunbar and Bose, 1991; Matsuda et al., 2014; Venkataraman et al., 2014; Weary et al., 2017]. Across mammals, the size of faecal particles usually increases with animal body mass [Fritz et al., 2009]. However, among primates, the proboscis monkey (*Nasalis larvatus*) displays a particularly small mean particle size (MPS) for its average body mass (15 kg) [Matsuda et al., 2014]. Proboscis monkeys are foregut fermenters [Matsuda et al., 2014]. Like other colobine primates, they have a sacculated forestomach where the food is fermented [Bauchop, and Martucci, 1968; Milton, 1993]. Regurgitation and remastication (i.e. rumination) has been observed in wild proboscis monkeys [Matsuda et al., 2011a]. Whether this facultative rumination strategy explains how proboscis monkeys achieve particularly fine faecal particles remains unclear [Matsuda et al., 2014].

Seasonal dietary change and dental wear are known to influence animals' chewing efficiency [Venkataraman et al., 2014]. Within a species, faecal particle size can vary in relation to diet [Renecker and Hudson, 1990] or seasons [Nygren and Hofmann, 1990]. For instance, in gelada baboons (*Theropithecus gelada*), chewing efficiency decreases during the dry season when individuals feed on tougher non-preferred food items, with a more distinct effect in older individuals [Venkataraman et al., 2014]. The opposite is observed in frugivorous chimpanzees (*Pan troglodytes schweinfurthii*): MPS is higher when chimpanzees feed on drupe fruits (preferred foods) than on figs (non-preferred foods) [Weary et al., 2017]. The authors suggested that chewing efficiency might be less critical in frugivores than in typical folivores, because they did not observe an effect of age on MPS.

With their natural diet, proboscis monkeys are excellent candidates to investigate how MPS might change throughout the year. While proboscis monkeys were first considered essentially folivores, it is now recognised that they preferentially feed on unripe fruits/seeds when they are available [Matsuda et al., 2009]. The present study investigates how proboscis monkeys' chewing efficiency varies among wet and dry seasons. We hypothesised that proboscis monkeys will achieve a higher chewing efficiency (MPS will decrease) during the season when individuals are able to consume their preferred food.

Moreover, we investigated some methodological aspects of sieve analysis. Extending the sieve column (adding larger top or smaller bottom sieves) is known to influence MPS measurements [Fritz et al., 2012], as well as including the weight of unchewed items (i.e. large seeds) and maximum particle length (MPL) in the MPS calculation [Weary et al., 2017]. Therefore, we combined various sets of sieves, with or without the MPL, and assessed the impact on MPS measurements.

## Material and Methods

### Study site

Our study took place in the Lower Kinabatangan Floodplain (5°20'-05°45 N, 117°40'-118°30' E), in Eastern Sabah (Malaysian Borneo), between 2015 and 2017. Daily temperatures and rainfall were measured at the research station. Below we will refer to dry season (May-June-July) where the mean monthly rainfall is 120 (± SD=100) mm and to the wet season (November-February) where it reaches 243 (±SD=104) mm. Mean minimum and maximum temperatures reached 24.4 (±SD=0.6) and 30 (±SD=1.7) °C, respectively.

### Faecal sampling

In riverine forests, proboscis monkeys are known to take refuge along riverbanks to spend the night [Matsuda et al., 2011b]. During this study, we conducted boat-based surveys along the Kinabatangan River, in the late afternoon, to find proboscis monkey groups settled at their sleeping sites. To avoid sampling the same group multiple times, we searched for proboscis monkey groups in different parts (North and South riverbanks) along a pre-established 21 km transect in a month. In the morning, we travelled back to the group's location of the previous evening. Once the group left the riverside to forage further inland, we moved to the riverbank to search for fresh faecal samples that had fallen under sleeping trees. We collected large samples, presumed to belong to adult individuals (undistinguished sex). Between May and July 2015, January and February 2016, and November 2016 and February 2017, two faecal samples were collected per group and placed in separate tubes, to perform two different analyses: manual and wet sieving analyses. 137 samples (15 ± 5 samples/month) were analysed by the manual method and 113 faecal samples (13 ± 6 samples/month) by wet sieving method.

### Manual analyses

Faecal samples were cleaned with water in a 0.4 mm mesh strainer to discard faecal matter. The remaining digested items were searched for intact seeds. Percentages of samples containing seeds were used to assess seasonal changes.

### Wet sieving analyses

Faecal samples were stored in a tube with 70% ethanol [Matsuda et al., 2014]. They were analysed using the standard wet sieving method [Fritz et al., 2012]. Before sieving, each sample was suspended in a beaker filled with water that was stirred continuously for 12 hours. The sample was then poured over a series of 10 sieves with mesh size of 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.63, 0.04 and 0.025 mm (Retsch AS 200 digit, Haan, Germany). We conducted the sieving analysis for 10 minutes with an amplitude of 2 mm and a water flow of approximately 2 l/min. If a particle was retained on the largest sieve, its size was recorded as the maximum particle size. Particles retained on each sieve were transferred onto pre-weighed petri dishes and dried at 103°C overnight. After cooling in a desiccator, petri dishes were weighed with an analysis balance with measuring accuracy of 1 mg (Kern AEJ 220-4M, Kern, Balingen, Germany). When large seeds (≥ 2 mm) were retained intact in sieves, they were removed, weighed and subtracted from the respective sieve weight. However, the smaller (< 2 mm) and numerous seeds, such as *Ficus* and *Nauclea* seeds, were logistically impossible to remove from the analysis [Weary et al., 2017].

Among various indices, the discrete mean has been proposed as a standard to describe the MPS value obtained from sieving analyses [Fritz et al., 2012]. To compare our results with a previous study conducted on proboscis monkey's faecal particle sizes [Matsuda et al., 2014], we excluded the two smallest sieves (mesh sizes: 0.040 and 0.025 mm) from the MPS calculation, as they were not used by Matsuda et al. [2014]. Although the latter study used a larger top sieve (16 mm) than we did, no particles were ever retained on it.

## Statistical analyses

We carried out t-tests on log-transformed data to compare $MPS_{(0.025\text{-}8mm)}$ values between dry and wet seasons. We compared MPS values calculated for 28 faecal samples, with or without considering the length of the maximum particles and using series of 10 (0.025-8 mm) or 8 (0.063-8 mm) sieves. To assess the difference between those four MPS measurements, we performed a related-samples Wilcoxon signed rank test between all pairs of MPS values, using the Bonferroni adjustment (for multiple comparisons). R 3.4.0 [R Development Core Team, 2016] was used for all statistical analyses, with statistical significance of $p < 0.05$.

## Results

### Manual analyses

By cleaning fresh faeces (N=137), we observed that the percentage of faecal samples containing intact seeds changed throughout the year (Fig. 1), with a mean of 100 ± 0 % during the wet season (November-February) and of 38 ± 10 % during the dry season (May-July).

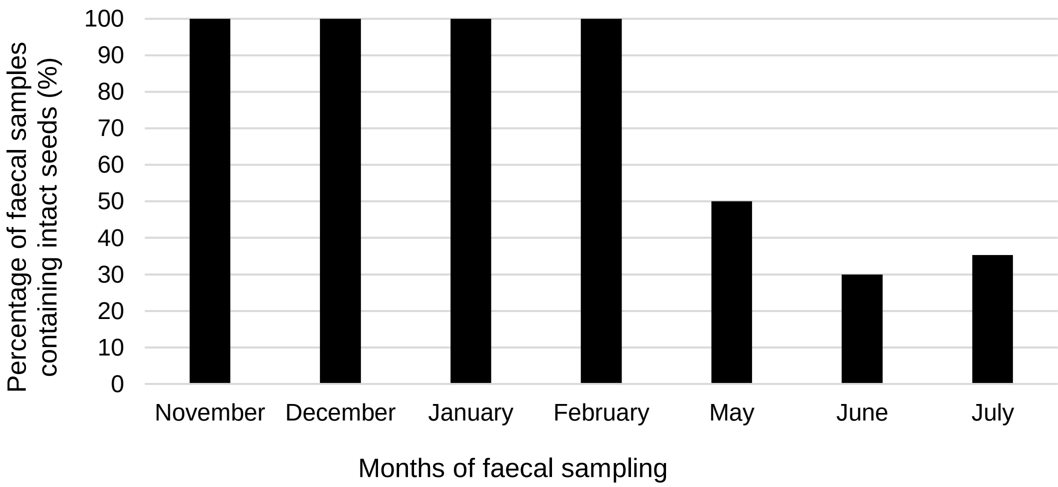


Figure 1 Percentage of faecal samples of proboscis monkeys (*Nasalis larvatus*) collected during different months that contain intact seeds

### Wet sieving analyses

Fig. 2 illustrates the typical way faecal particles are distributed after wet sieving analysis.

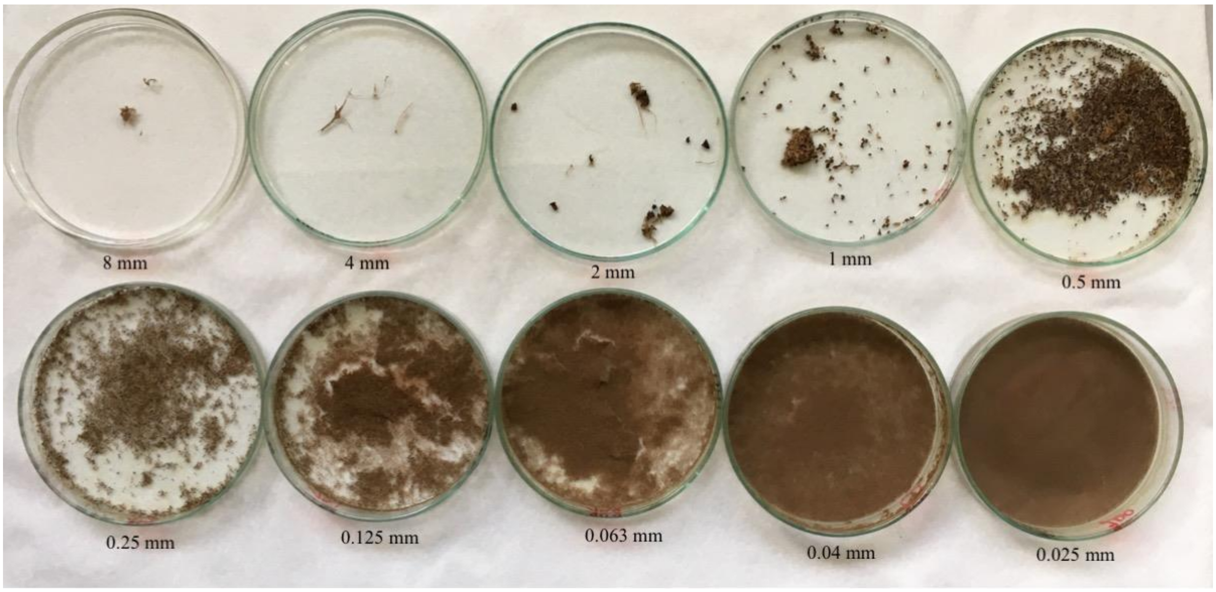


Figure 2. Distribution of proboscis monkey (*Nasalis larvatus*) faecal particles after wet sieving analysis. Each petri dish represents one of the 10 cascade sieves, ordered from 8 mm until 0.025 mm mesh size. The sample PMF217-1 collected on the 28th of January 2017 was used for this example.

By using a cascade of 10 sieves (mesh sizes: 8 to 0.025 mm), the MPS reaches an average of 0.45 ± 0.14 mm and increases over the course of the observation period from November to July (Fig. 3). By using a series of eight sieves (mesh sizes: 8 to 0.063 mm) like in Matsuda et al. (2014), we observe that $MPS_{(0.063\text{-}8mm)}$ is significantly larger than $MPS_{(0.025\text{-}8mm)}$, reaching 0.55 ± 0.14 mm ($V=6441$, $N=113$, $p<0.001$).

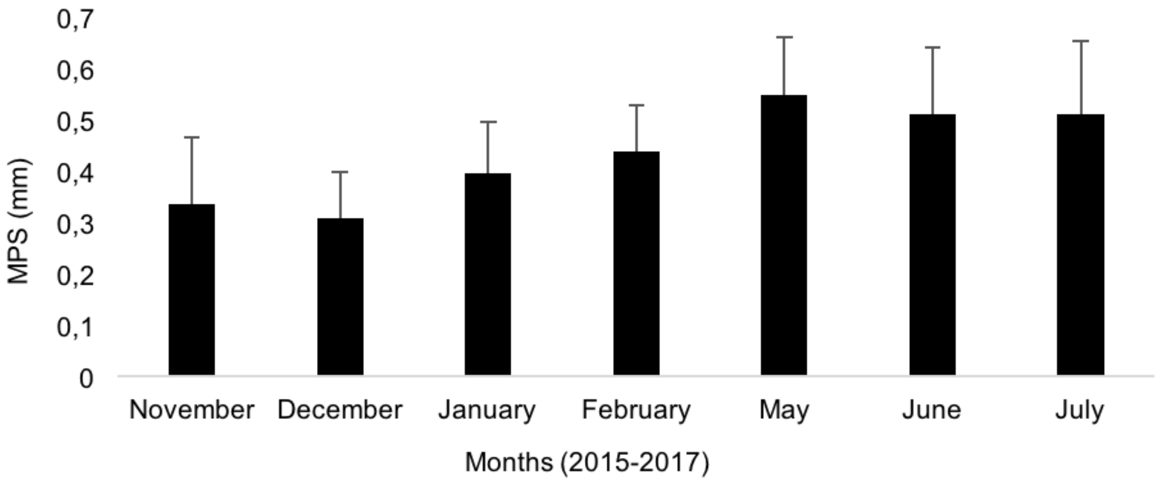


Figure 3. Variation in proboscis monkey (*Nasalis larvatus*) MPS over the course of the observation period (November-July)

When comparing $MPS_{(0.025\text{-}8mm)}$ of proboscis monkeys across seasons, we observe that MPS is significantly smaller during the wet season than the dry season ($MPS_{wet} = 0.38 \pm 0.11$ mm, $MPS_{dry} = 0.52 \pm 0.13$ mm; t-test $t = -6.2812$, $p < 0.001$) (Fig. 4).

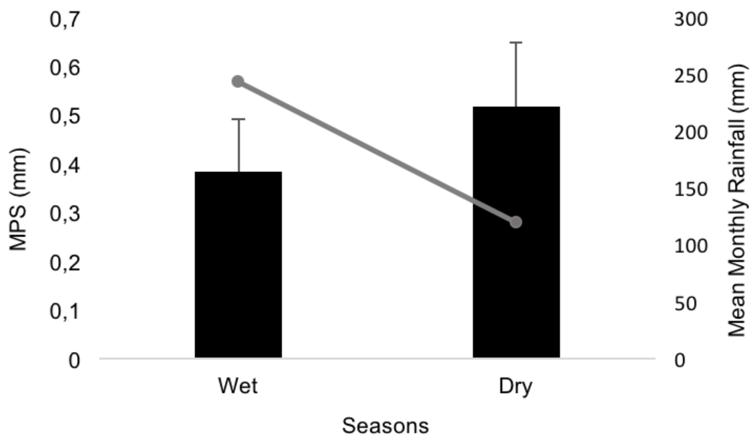


Figure 4. MPS variation of proboscis monkeys (*Nasalis larvatus*) across seasons

Table 1 summarises four MPS measurements. Using a series of 10 sieves (with both smallest mesh sizes 0.040 and 0.025 mm) resulted in finer MPS than calculation with the 8-sieve cascade. MPS measurements were significantly larger when including the maximum particle length (MPL) for the largest sieve in the calculation.

Table 1. Mean particle size ± SD of 28 faecal samples of proboscis monkeys (*Nasalis larvatus*) measured using two series of sieves (indicated by the sieve size of the smallest and largest sieves) and with or without taking the maximum particle length (MPL, when particles were retained on the largest sieve) into account in the MPS calculation

| Method | MPS ± SD (mm) |
|---|---|
| 0.025-8 MPL | 0.48 ± 0.11 |
| 0.025-8 | 0.47 ± 0.11 |
| 0.063-8 MPL | 0.59 ± 0.11 |
| 0.063-8 | 0.57 ± 0.10 |

**Discussion**

This study focused on proboscis monkeys' faecal samples collected between 2015 and 2017, during wet and dry seasons. We confirm the very small discrete mean faecal particle size in proboscis monkey ($MPS_{(0.025\text{-}8mm)}$ = 0.45 ± 0.14 mm), for its average body mass. We measured a $MPS_{(0.063\text{-}8mm)}$ of 0.55 ± 0.14 mm, similar to the results of a previous study ($MPS_{(0.063\text{-}16mm)}$ = 0.53 ± 0.09 mm) obtained by analysing 10 samples collected in June-July 2010 [Matsuda et al., 2014]. The fine MPS indicates the generally high chewing efficiency of proboscis monkeys. For example, in comparison, frugivorous primates in Borneo, such as macaques (*Macaca fascicularis* and *M. nemestrina*) and orangutans (*Pongo pygmaeus*), have larger $MPS_{(0.063\text{-}16mm)}$ respectively ranging from 1.07 ± 0.47 to 2.30 ± 0.78 mm [Matsuda et al., 2014].

We observed that MPS is even smaller during the wet season than the dry season. The same pattern had been observed in folivorous gelada baboons [Venkataraman et al., 2014]. The latter showed a lower MPS in wet season when consuming less tough food items. In our study, the MPS difference might also be linked to a change in diet. Smaller MPS values during the wet season correlate with high percentage of intact seeds in faeces, suggesting individuals might consume more fruits and their seeds. However, this assumption must be considered carefully, as the absence of seeds in faeces does not always imply that individuals did not eat fruits (i.e. seeds could be totally digested, chewed or discharged). Mismatches have been observed between proboscis monkey fruit feeding activity and seeds detected in faeces [Matsuda et al., 2013]. Primates, including proboscis monkeys, usually avoid feeding on tough leaves or leaf parts [Hill and Lucas, 1996; Teaford et al., 2006; Dunham and Lambert, 2016; Matsuda et al., 2017]. The same pattern is observed in chimpanzees where fallback foods are significantly tougher than preferred items (fruits) [Vogel et al., 2008]. However, in Bornean orangutans (*P. p. wurmbii*), mechanical properties of leaves and fruits did not vary significantly [Vogel et al., 2008]. In the present study, we did not analyse the toughness of food items fed on by proboscis monkeys. However, in comparison to leaves, fruits and seeds are generally considered as high quality food [Milton, 1993; Hanya and Bernard, 2015]. Containing less fibre, fruits generally are more digestible than leaves [Milton, 1993]; consumption of unripe fruits may lead to smaller MPS. Further work should investigate nutritional and mechanical properties of unripe fruits and their seeds consumed by the proboscis monkey to better understand the feeding selection in this endangered primate.

As in gelada baboons, we suggest here that fallback food consumption during some parts of the year leads to a reduction of chewing efficiency which might potentially negatively impact the animals' fitness [Venkataraman et al., 2014]. There is preliminary evidence that 'rumination' activity in proboscis monkeys is higher during times of increased leaf consumption [Matsuda et al., 2014], which could potentially attenuate the change in MPS associated with leaves. If this was a general pattern, then the MPS difference between the seasons obtained in the present study should be of a lower magnitude than in other arboreal primates that show a seasonal foraging pattern but do not 'ruminate'. Compared to geladas with a seasonal MPS difference of 0.3-0.4 mm in prime adults, the proboscis monkeys of the present study did show a lower difference (0.14 mm, Fig. 4). However, due to diet differences between hindgut fermenter geladas and foregut fermenter proboscis monkeys, this comparison should be treated cautiously. We suggest further studies should determine whether proboscis monkey individuals achieve finer MPS when they ‘ruminate’ as opposed to times when they do not. Such data could help unravel the relevance of facultative rumination as a response to diet constraints. Finally, future research should also investigate if seasonal changes in MPS are also measured in other colobine primates, as data is missing so far.


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