1   **Title:**

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

3   seasons

4

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28 **Abstract**

29 Reducing the size of food particles is crucial for herbivores. Seasonal dietary changes are

30 known to influence animals' chewing efficiency. Proboscis monkeys (*Nasalis larvatus*) are

31 foregut fermenters, with a high chewing efficiency allowing them to achieve very fine faecal

32 particles. In this study, we investigated how proboscis monkeys' chewing efficiency varies

33 among wet and dry seasons, hypothesising differences possibly related to diet change. Faecal

34 particle size analysis is an established approach to estimate chewing efficiency in mammalian

35 herbivores. We analysed 113 proboscis monkey faecal samples collected in the Lower

36 Kinabatangan Wildlife Sanctuary, between 2015 and 2017. By following standard sieve

37 analysis protocols, we measured a mean particle size $MPS_{(0.025-8)}$ of 0.45 ± 0.14 mm, and

38 confirmed a previous result that proboscis monkeys have a very low faecal MPS. This study

39 highlights a seasonal influence on proboscis monkeys' chewing efficiency, with smaller MPS

40 (better chewing efficiency) during the wet season. During that time of the year, individuals

41 may potentially change their diet, as all faecal samples contained intact seeds. Whether the

42 seasonal MPS difference in proboscis monkeys is smaller than in other colobines due to their

43 'rumination' strategy remains to be investigated.

44

45 **Keywords**

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

47 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

76   frugivorous chimpanzees (*Pan troglodytes schweinfurthii*): MPS is higher when chimpanzees

77   feed on drupe fruits (preferred foods) than on figs (non-preferred foods) [Weary et al., 2017].

78   The authors suggested that chewing efficiency might be less critical in frugivores than in

79   typical folivores, because they did not observe an effect of age on MPS.

80   With their natural diet, proboscis monkeys are excellent candidates to investigate how

81   MPS might change throughout the year. While proboscis monkeys were first considered

82   essentially folivores, it is now recognised that they preferentially feed on unripe fruits/seeds

83   when they are available [Matsuda et al., 2009]. The present study investigates how proboscis

84   monkeys' chewing efficiency varies among wet and dry seasons. We hypothesised that

85   proboscis monkeys will achieve a higher chewing efficiency (MPS will decrease) during the

86   season when individuals are able to consume their preferred food.

87   Moreover, we investigated some methodological aspects of sieve analysis. Extending

88   the sieve column (adding larger top or smaller bottom sieves) is known to influence MPS

89   measurements [Fritz et al., 2012], as well as including the weight of unchewed items (i.e.

90   large seeds) and maximum particle length (MPL) in the MPS calculation [Weary et al., 2017].

91   Therefore, we combined various sets of sieves, with or without the MPL, and assessed the

92   impact on MPS measurements.

93

94   **Material and Methods**

95   Study site

96   Our study took place in the Lower Kinabatangan Floodplain (5°20'-05°45 N, 117°40'-

97   118°30' E), in Eastern Sabah (Malaysian Borneo), between 2015 and 2017. Daily

98   temperatures and rainfall were measured at the research station. Below we will refer to dry

99   season (May-June-July) where the mean monthly rainfall is 120 ($\pm$ SD=100) mm and to the

100   wet season (November-February) where it reaches 243 (±SD=104) mm. Mean minimum and

101   maximum temperatures reached 24.4 (±SD=0.6) and 30 (±SD=1.7) °C, respectively.

102

103   Faecal sampling

104   In riverine forests, proboscis monkeys are known to take refuge along riverbanks to spend the

105   night [Matsuda et al., 2011b]. During this study, we conducted boat-based surveys along the

106   Kinabatangan River, in the late afternoon, to find proboscis monkey groups settled at their

107   sleeping sites. To avoid sampling the same group multiple times, we searched for proboscis

108   monkey groups in different parts (North and South riverbanks) along a pre-established 21 km

109   transect in a month. In the morning, we travelled back to the group's location of the previous

110   evening. Once the group left the riverside to forage further inland, we moved to the riverbank

111   to search for fresh faecal samples that had fallen under sleeping trees. We collected large

112   samples, presumed to belong to adult individuals (undistinguished sex). Between May and

113   July 2015, January and February 2016, and November 2016 and February 2017, two faecal

114   samples were collected per group and placed in separate tubes, to perform two different

115   analyses: manual and wet sieving analyses. 137 samples (15 ± 5 samples/month) were

116   analysed by the manual method and 113 faecal samples (13 ± 6 samples/month) by wet

117   sieving method.

118

119   Manual analyses

120   Faecal samples were cleaned with water in a 0.4 mm mesh strainer to discard faecal matter.

121   The remaining digested items were searched for intact seeds. Percentages of samples

122   containing seeds were used to assess seasonal changes.

123

124   Wet sieving analyses

125 Faecal samples were stored in a tube with 70% ethanol [Matsuda et al., 2014]. They were

126 analysed using the standard wet sieving method [Fritz et al., 2012]. Before sieving, each

127 sample was suspended in a beaker filled with water that was stirred continuously for 12 hours.

128 The sample was then poured over a series of 10 sieves with mesh size of 8, 4, 2, 1, 0.5, 0.25,

129 0.125, 0.63, 0.04 and 0.025 mm (Retsch AS 200 digit, Haan, Germany). We conducted the

130 sieving analysis for 10 minutes with an amplitude of 2 mm and a water flow of approximately

131 2 l/min. If a particle was retained on the largest sieve, its size was recorded as the maximum

132 particle size. Particles retained on each sieve were transferred onto pre-weighed petri dishes

133 and dried at 103°C overnight. After cooling in a desiccator, petri dishes were weighed with an

134 analysis balance with measuring accuracy of 1 mg (Kern AEJ 220-4M, Kern, Balingen,

135 Germany). When large seeds ($\geq$ 2 mm) were retained intact in sieves, they were removed,

136 weighed and subtracted from the respective sieve weight. However, the smaller (< 2 mm) and

137 numerous seeds, such as *Ficus* and *Nauclea* seeds, were logistically impossible to remove

138 from the analysis [Weary et al., 2017].

139

140 Among various indices, the discrete mean has been proposed as a standard to describe the

141 MPS value obtained from sieving analyses [Fritz et al., 2012]. To compare our results with a

142 previous study conducted on proboscis monkey's faecal particle sizes [Matsuda et al., 2014],

143 we excluded the two smallest sieves (mesh sizes: 0.040 and 0.025 mm) from the MPS

144 calculation, as they were not used by Matsuda et al. [2014]. Although the latter study used a

145 larger top sieve (16 mm) than we did, no particles were ever retained on it.

146

147   Statistical analyses

148 We carried out t-tests on log-transformed data to compare $MPS_{(0.025-8mm)}$ values between dry

149 and wet seasons. We compared MPS values calculated for 28 faecal samples, with or without

150   considering the length of the maximum particles and using series of 10 (0.025-8 mm) or 8

151   (0.063-8 mm) sieves. To assess the difference between those four MPS measurements, we

152   performed a related-samples Wilcoxon signed rank test between all pairs of MPS values,

153   using the Bonferroni adjustment (for multiple comparisons). R 3.4.0 [R Development Core

154   Team, 2016] was used for all statistical analyses, with statistical significance of $p < 0.05$.

155

156   **Results**

157   Manual analyses

158   By cleaning fresh faeces (N=137), we observed that the percentage of faecal samples

159   containing intact seeds changed throughout the year (Fig. 1), with a mean of 100 ± 0 % during

160   the wet season (November-February) and of 38 ± 10 % during the dry season (May-July).
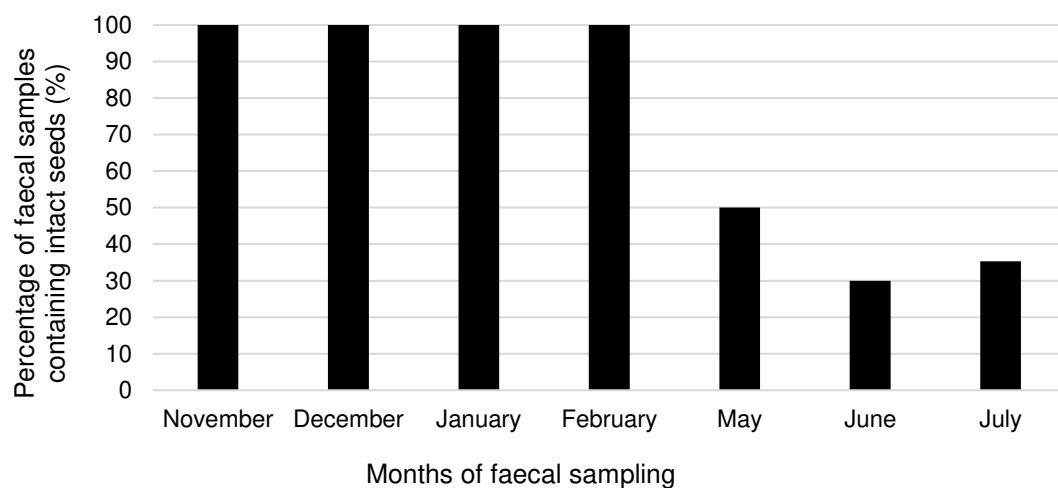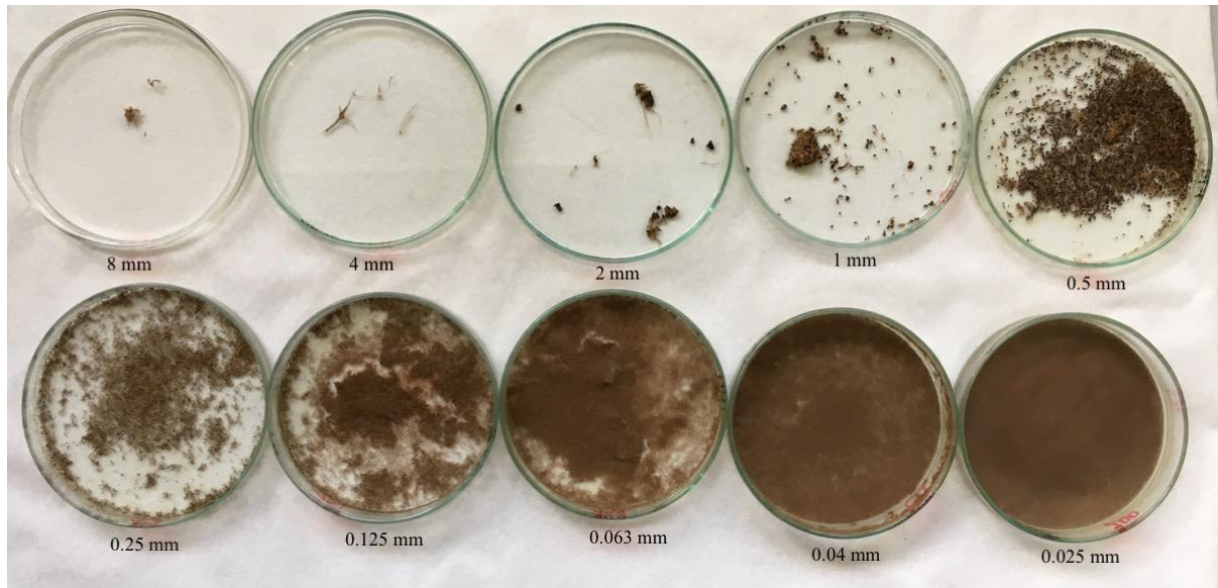

161

162   Figure 1 Percentage of faecal samples of proboscis monkeys (*Nasalis larvatus*) collected

163   during different months that contain intact seeds

164

165   Wet sieving analyses

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

167

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).
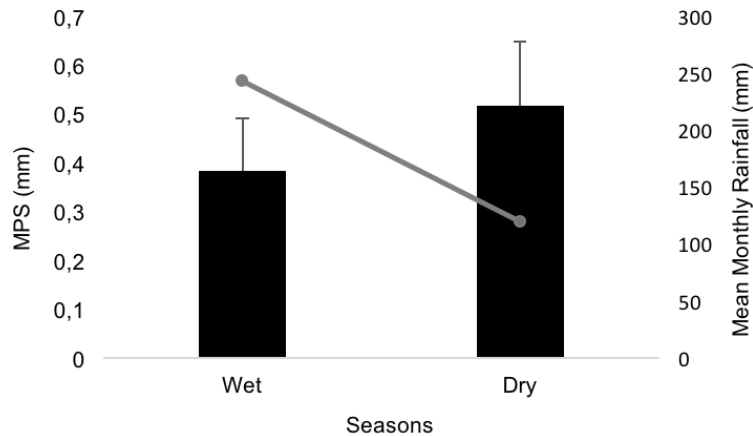
179
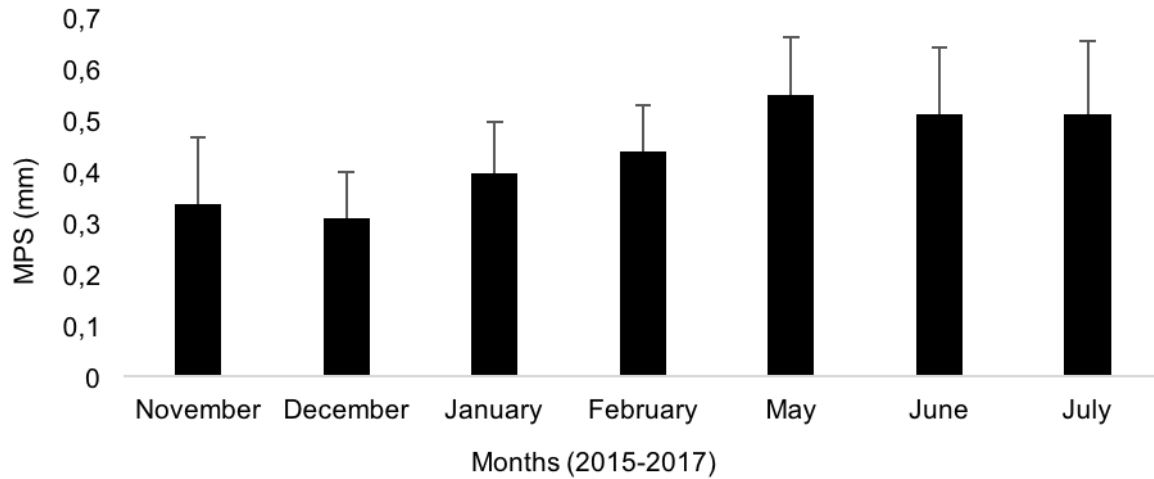
Figure 3. Variation in proboscis monkey (*Nasalis larvatus*) MPS over the course of the observation period (November-July)

When comparing $MPS_{(0.025-8mm)}$ 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).

186

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.

193

194 Table 1. Mean particle size ± SD of 28 faecal samples of proboscis monkeys (*Nasalis*

195 *larvatus*) measured using two series of sieves (indicated by the sieve size of the smallest and

196 largest sieves) and with or without taking the maximum particle length (MPL, when particles

197 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

198

199 **Discussion**

200   This study focused on proboscis monkeys' faecal samples collected between 2015 and

201 2017, during wet and dry seasons. We confirm the very small discrete mean faecal particle

202 size in proboscis monkey ($MPS_{(0.025-8mm)}$ = 0.45 ± 0.14 mm), for its average body mass. We

203 measured a $MPS_{(0.063-8mm)}$ of 0.55 ± 0.14 mm, similar to the results of a previous study

204 ($MPS_{(0.063-16mm)}$ = 0.53 ± 0.09 mm) obtained by analysing 10 samples collected in June-July

205 2010 [Matsuda et al., 2014]. The fine MPS indicates the generally high chewing efficiency of

206 proboscis monkeys. For example, in comparison, frugivorous primates in Borneo, such as

207 macaques (*Macaca fascicularis* and *M. nemestrina*) and orangutans (*Pongo pygmaeus*), have

208 larger $MPS_{(0.063-16mm)}$ respectively ranging from 1.07 ± 0.47 to 2.30 ± 0.78 mm [Matsuda et

209 al., 2014].

210   We observed that MPS is even smaller during the wet season than the dry season. The

211 same pattern had been observed in folivorous gelada baboons [Venkataraman et al., 2014].

212 The latter showed a lower MPS in wet season when consuming less tough food items. In our

213 study, the MPS difference might also be linked to a change in diet. Smaller MPS values

214 during the wet season correlate with high percentage of intact seeds in faeces, suggesting

215 individuals might consume more fruits and their seeds. However, this assumption must be

216 considered carefully, as the absence of seeds in faeces does not always imply that individuals

217 did not eat fruits (i.e. seeds could be totally digested, chewed or discharged). Mismatches

218 have been observed between proboscis monkey fruit feeding activity and seeds detected in

219 faeces [Matsuda et al., 2013]. Primates, including proboscis monkeys, usually avoid feeding

220 on tough leaves or leaf parts [Hill and Lucas, 1996; Teaford et al., 2006; Dunham and

221 Lambert, 2016; Matsuda et al., 2017]. The same pattern is observed in chimpanzees where

222 fallback foods are significantly tougher than preferred items (fruits) [Vogel et al., 2008].

223 However, in Bornean orangutans (*P. p. wurmbii*), mechanical properties of leaves and fruits

224 did not vary significantly [Vogel et al., 2008]. In the present study, we did not analyse the

225 toughness of food items fed on by proboscis monkeys. However, in comparison to leaves,

226 fruits and seeds are generally considered as high quality food [Milton, 1993; Hanya and

227 Bernard, 2015]. Containing less fibre, fruits generally are more digestible than leaves [Milton,

228 1993]; consumption of unripe fruits may lead to smaller MPS. Further work should

229 investigate nutritional and mechanical properties of unripe fruits and their seeds consumed by

230 the proboscis monkey to better understand the feeding selection in this endangered primate.

231 As in gelada baboons, we suggest here that fallback food consumption during some

232 parts of the year leads to a reduction of chewing efficiency which might potentially negatively

233 impact the animals' fitness [Venkataraman et al., 2014]. There is preliminary evidence that

234 'rumination' activity in proboscis monkeys is higher during times of increased leaf

235 consumption [Matsuda et al., 2014], which could potentially attenuate the change in MPS

236 associated with leaves. If this was a general pattern, then the MPS difference between the

237 seasons obtained in the present study should be of a lower magnitude than in other arboreal

238 primates that show a seasonal foraging pattern but do not 'ruminate'. Compared to geladas

239 with a seasonal MPS difference of 0.3-0.4 mm in prime adults, the proboscis monkeys of the

240 present study did show a lower difference (0.14 mm, Fig. 4). However, due to diet differences

241 between hindgut fermenter geladas and foregut fermenter proboscis monkeys, this

242 comparison should be treated cautiously. We suggest further studies should determine

243 whether proboscis monkey individuals achieve finer MPS when they 'ruminate' as opposed to

244 times when they do not. Such data could help unravel the relevance of facultative rumination

245 as a response to diet constraints. Finally, future research should also investigate if seasonal

246 changes in MPS are also measured in other colobine primates, as data is missing so far.

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