1 **Activity budget and gut microbiota across reproductive states in wild, female capuchin**

2 **monkeys in a seasonal dry forest**

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14 **ABSTRACT**

15

16 Energy demands associated with pregnancy and lactation are significant forces in mammalian

17 evolution. To mitigate increased energy costs associated with reproduction, female mammals

18 have evolved behavioral and physiological responses. Some species alter activity to conserve

19 energy during pregnancy and lactation, while others experience changes in metabolism and fat

20 deposition. Restructuring of gut microbiota with shifting reproductive states may also help

21 females increase energy harvest from foods, especially during pregnancy. Here, we combine life

22 history data with >13,000 behavioral scans and >300 fecal samples collected longitudinally

23 across multiple years from 33 white-faced capuchin monkey females to examine the

24 relationships among behavior, gut microbiota composition, and reproductive state. We used 16S-

25 based amplicon sequencing and the DADA2 pipeline to analyze microbial diversity and putative

26 functions. Reproductive state explained some variation in activity, but overall resting and

27 foraging behaviours were relatively stable across the reproductive cycle. We found evidence for

28 increases in biotin synthesis pathways among microbes in lactating females, and that microbial

29 community dissimilarity among the states was small but significant. Otherwise, gut microbiota

30 structure and estimated functions were not substantially different among reproductive states.

31   These data contribute to a broader understanding of plasticity in response to physiological shifts

32   associated with mammalian reproduction.

33

34   **INTRODUCTION**

35   The demands of pregnancy and lactation have been an influential force throughout mammalian

36   evolution. Female mammals experience discrete stages of the reproductive cycle, including

37   cycling, pregnancy, and lactation, but variation across mammalian taxa exists in response to

38   cycling parameters, litter size, birth weight, gestation length, weaning age, weaning mass, and

39   interbirth interval (Gittleman & Thompson, 1988). Lactation is typically the most energetically

40   demanding stage of the reproductive cycle because milk production and other aspects of infant

41   care, incuding infant carrying, require considerable energy above basal metabolic function

42   (Clutton-Brock et al., 1989; Dewey, 1997; Gittleman & Thompson, 1988). Pregnancy is the

43   second most energetically-demanding state, and non-pregnant, non-lactation states (i.e. cycling

44   and non-cycling pauses) are the least energetically costly (Dufour & Sauther, 2002; Serio-Silva

45   et al., 1999). In addition to heightened net energy demands, pregnancy and lactation also

46   introduce increased protein and other nutrient requirements to fuel fetal and infant growth

47   (Dewey, 1997; National Research Council, 2003). Energy requirements typically increase as a

48   fetus develops during pregnancy; after parturition, energy demands continue to increase as the

49   mother produces milk (Ellison, 2003; Emery Thompson, 2013; Villar et al., 1992). As the infant

50   grows and needs more milk combined with larger infant size, energy demand on the mother

51   continues to grow. During the final stages of lactation, once the infant becomes semi-

52   independent in the lead-up to weaning, energy requirements related to infant care decrease

53   (Figure 1).

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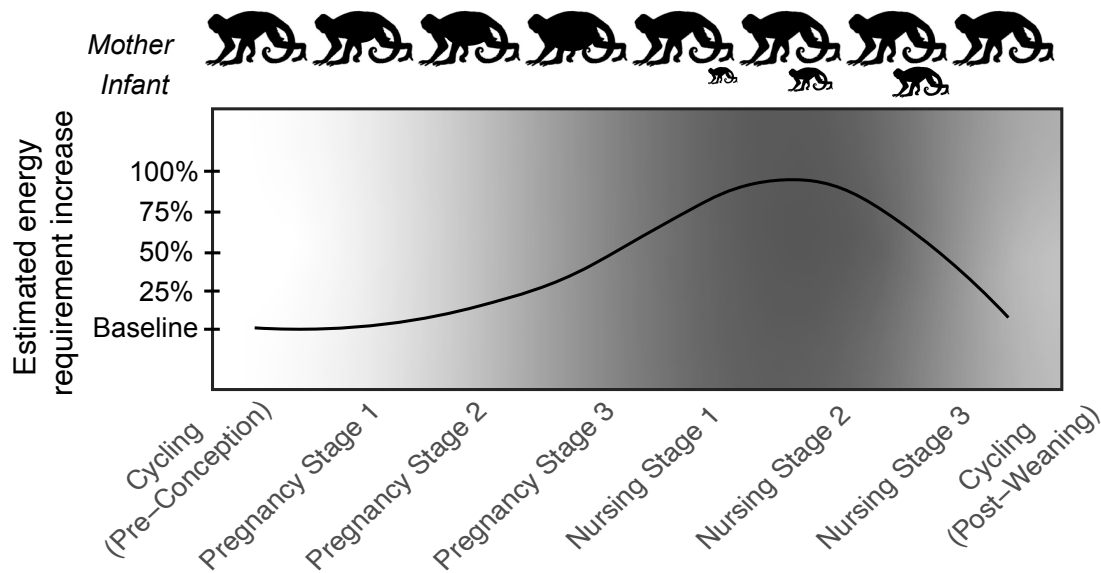
Figure 1. Visualization of estimated increases in energy requirements during the reproductive cycle of a non-human primate. Female primates face a 25% increase in daily energy requirements during gestation, and up to a 50-100% increase during lactation (Key & Ross, 1999).

Mammals vary widely in the length and energy costs of reproduction and have evolved multiple strategies in response. Adaptations include behavioral responses to seasonal fluctuations in food availability. For example, harbour seals (*Phoca vitulina*) and other migratory species travel to specific breeding sites and feeding sites, and exhibit strictly seasonal breeding that is tied to food abundance in their environment (Bowen et al., 2001). For mammals that are not constrained by migratory patterns or strict seasonal breeding, behavioral flexibility—including modulating energy expenditure, foraging rates, and food choice—offers a strategy to mitigate increased energy costs of pregnancy and lactation. Primates, including humans, exemplify these behvioral strategies. While some primates conserve energy during costly reproductive stages by resting for larger proportions of the day (e.g. red-ruffed lemurs [*Varecia rubra*], (Vasey, 2005); green monkeys [*Cercopithecus sabaeus*] (Harrison, 1983)), others increase energy intake, either by foraging for longer periods of the day (e.g. yellow baboons (*Papio cynocephalus*) (Altmann,

75   2001)) or by increasing their intake rate of foods (e.g. white-faced capuchins [*Cebus capucinus*

76   *imitator*] (McCabe & Fedigan, 2007)).

77   Adaptations to the demands of the reproductive cycle also include physiological changes that

78   occur within the mother. For example, changing patterns of fat gain enable females to

79   accumulate fat stores during pregnancy that they can draw from during lactation. Humans

80   typically experience increased fat deposition during pregnancy, even in cases when they are

81   experiencing food stress (Poppitt et al., 1993). Similar results were found in a study of captive

82   bonobos (*Pan paniscus*), in which pregnant females did not lose weight while experiencing

83   caloric restriction (Deschner et al., 2008). Sufficient temporary fat gain during pregnancy

84   supports efficient and healthy development of infants. Too little fat gain may lead to increased

85   periods of lactation and increased interbirth interval (e.g. humans (Lunn et al., 1984)), while too

86   much fat gain during gestation can lead to birth complications (e.g. domestic canines and felines

87   (Fontaine, 2012)).

88   Research on humans suggests that pregnancy is also associated with changes in gut microbial

89   communities (DiGiulio et al., 2015; Koren et al., 2012; Smid et al., 2018). These changes, which

90   include reduced diversity of microbes, shifts in prominent bacterial phyla associated with energy

91   harvest, and shifts in putative metabolic pathways related to energy absorption are linked to

92   metabolic disease states in non-pregnant individuals. However, in the context of reproductive

93   demands, they may serve an adaptive role in increasing energy harvest from food during times of

94   increased energy need for fetal development and allow for increased fat storage to cope with

95   costs of lactation (Edwards et al., 2017; Koren et al., 2012). In non-human mammals, evidence

96   suggests gut microbiota change during reproduction (e.g. Tibetan macaques (Sun et al., 2021)),

97   and shifts may be hormonally mediated (Mallott et al., 2020). However, other researchers have

98   found that composition and predicted function of individuals' gut microbiota remained relatively

99   static throughout pregnancy and into early lactation (Jost et al., 2013). These contrasting findings

100   may be due to differences in study design, methods, and sample species and population.

101   Alternatively, they may indicate that the degree to which the gut microbiome can shift during

102   pregnancy is constrained or enabled by external factors.

4

103   Behavioral and gut microbial changes might interact to address the demands of pregnancy and

104   lactation. However, few studies have combined behavioral and gut microbial data tracked

105   throughout pregnancy and lactation to understand the nuances of how mammals in a wild setting

106   cope with increased energy requirements. Here, we combine behavioral and gut microbial data

107   from a well-studied population of wild non-human primates to examine the strategy or

108   combination of strategies that female primates employ to address the increased energetic costs of

109   pregnancy and lactation. To examine behavioral and gut microbial community flexibility as they

110   relate to the reproductive cycle, we studied a population of omnivorous, wild white-faced

111   capuchin monkeys that exhibit moderately seasonal breeding. Specifically, we examine white-

112   faced capuchin monkey responses to changing reproductive stages over the course of 5 years in a

113   seasonal dry forest. We combine a robust data set of >13000 behavioral scans with >300 fecal

114   samples collected from 33 monkeys to study behavioral and gut microbial responses to

115   reproduction in a species that inhabits a dynamic and seasonal ecosystem. Our first aim was to

116   compare activity budgets of white-faced capuchins among and within cycling, pregnancy, and

117   nursing stages. We predict that if capuchins employ an "energy conservation" approach during

118   pregnancy and nursing, then females will rest more in stages of higher energy demand compared

119   to stages of lower energy demand. Conversely, if capuchins employ an "energy maximization"

120   approach during pregnancy and lactation, then females will forage for larger proportions of their

121   day compared to cycling capuchins. Our second aim was to investigate gut microbial changes in

122   female capuchins among cycling, pregnant, and nursing states. We predict that gut microbiota

123   will exhibit characteristics associated with increased capacity for energy harvest during periods

124   of highest energy demand during pregnancy. We also predict that females' gut microbiota will

125   exhibit an increase in relative abundance of putative metabolic pathways related to energy

126   metabolism and carbohydrate transport during pregnancy. Given the demonstrated potential for

127   ecological and social factors to influence behavioral or gut microbial flexibility in this species,

128   we additionally examine the potential effects of precipitation, temperature, diet, fruit biomass in

129   the environment, and dominance rank on activity budget and gut microbial communities.

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132

133   **RESULTS**

134

135   *Aim 1:* **Compare activity budgets of white-faced capuchins among and within cycling,**

136   **pregnancy, and nursing stages**

137

138   To visualize overall activity budget shifts across the reproductive cycle, we combined related

139   behaviors (see Ethogram, Supplemental Table 1) into six general categories: Foraging, Resting,

140   Social Affiliation, Social Aggression, Travel, and Other. We calculated proportions of each

141   category per total scans per day (Figure 2).

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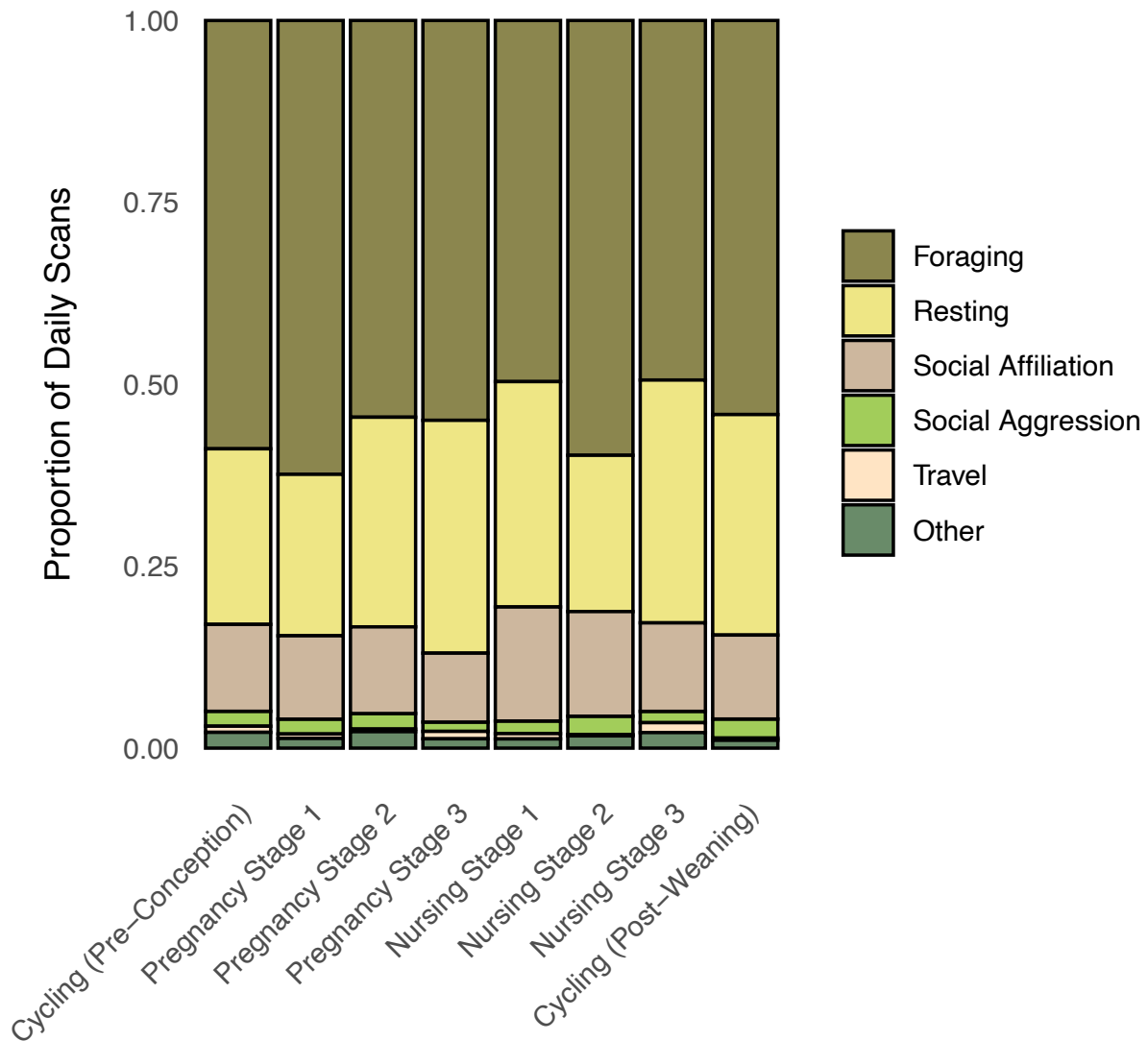
142 **Figure 2**. Proportions of daily scans spent in each of six behavioral categories across the

143 reproductive cycle. These data represent raw counts of scans per behavior per day divided by

144 total scans recorded per day.

145

146 *Resting activity within and among reproductive states*

147 A generalized linear mixed model of resting activity that included reproductive state

148 outperformed a null model excluding this variable, suggesting some variation in resting behavior

149 was explained by reproductive stage. High social rank was significantly negatively related to

150 total resting scans (Estimate = -0.13, SE = 0.06, Z-Value = -2.16, p = 0.03), indicating that

151 higher ranking individuals rested less often than lower or mid-ranking individuals. Maximum

152 temperature was significantly positively related to total resting scans indicating that monkeys

153 rested more often in hot temperatures (Estimate = 0.22, SE = 0.02, Z-Value = 10.35, p < 0.001)

154 (Supplemental Table 2). Incident Rate Ratios for all predictors are presented in Figure 3a and

155 values reported in Supplemental Table 2. Predicted counts of resting scans per day are visualized

156 in Figure 3b and demonstrate that resting increased throughout pregnancy and early nursing,

157 dipped in mid-nursing, and increased again in late nursing. However, variation was minor and

158 we did not find significant pairwise differences among the eight individual reproductive stages.
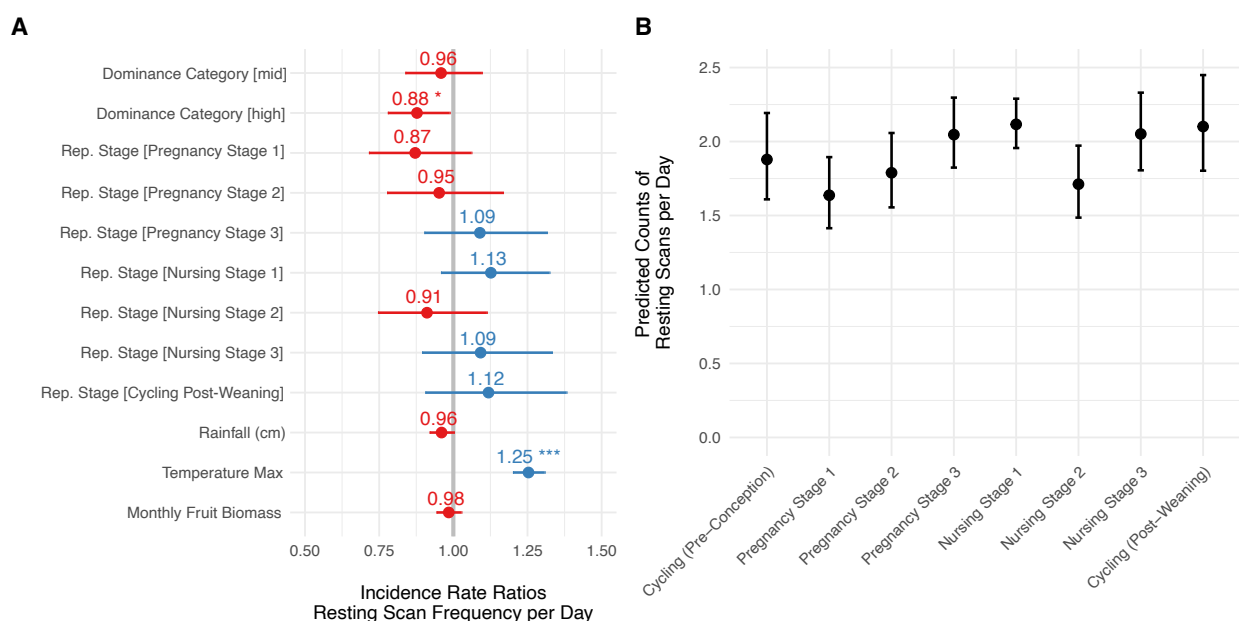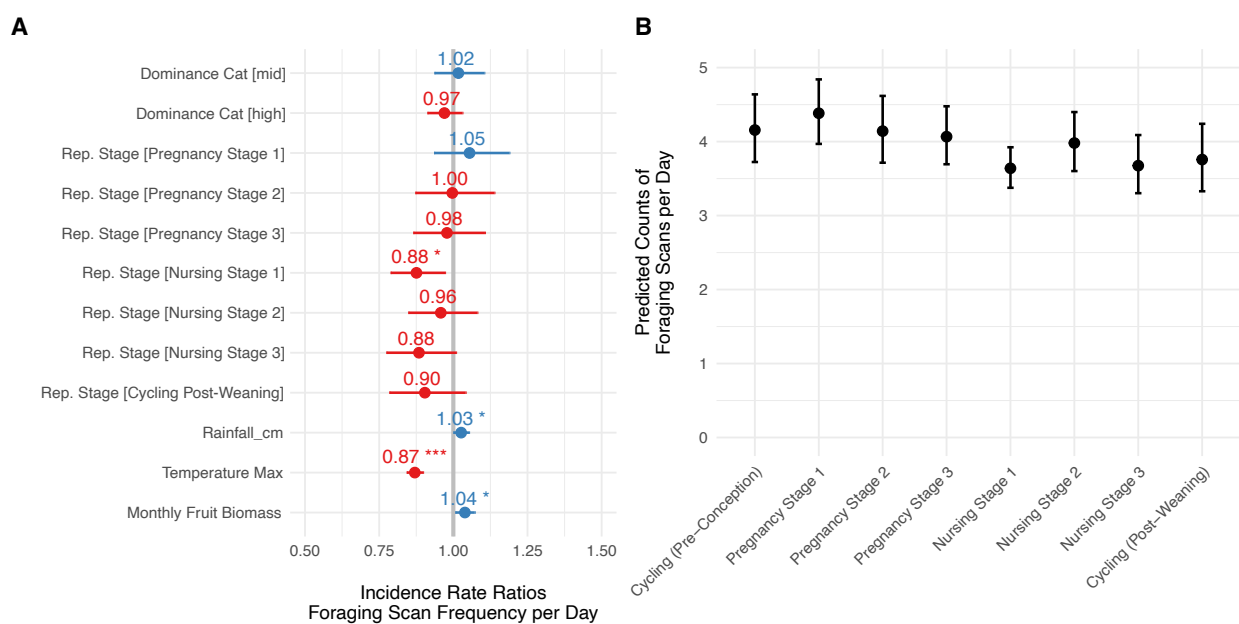

159

160   **Figure 3**. Incidence rate ratios (IRR) and standard error (**A**) for predictors from a GLMM of

161   resting scans per day. The reference dominance category is low social rank; the reference

162   reproductive stage is cycling (pre-conception). The grey vertical line represents "no effect".

163   Values to the right of the grey line represent positive effects and values to the left represent

164   negative effects. Significant predictors ($p < 0.05$) are denoted with asterisks. We present an

165   alternative way to visualise the effect of reproductive stage on resting activity by plotting the

166   predicted number of resting scans per day for each level of reproductive stage variable (**B**).

167   *Foraging activity within and among reproductive states*

168

169   The generalized linear mixed model of foraging activity that included reproductive state

170   outperformed a null model excluding this variable. Females in Nursing Stage 1 exhibited

171   significantly fewer foraging scans per day compared to other stages (Estimate = -0.13, SE = 0.05,

172   Z-Value = -2.45, p = 0.01). Ecological variables including rainfall, daily maximum temperature,

173   and estimates fruit biomass were also significantly correlated with foraging scans per day and

174   values are reported in Supplemental Table 2). Incident Rate Ratios for all predictors in the model

175   are presented in Figure 4a and we visualized predicted counts of foraging scans per day in Figure

176   4b. These predicted counts, which take into account all other predictors in the foraging model,

177   suggest that foraging scans steadily decreased throughout pregnancy and into early nursing

178   before increasing throughout late nursing and into post-weaning cycling.

179

Figure 4. Incidence rate ratios (IRR) and standard error (**A**) for predictors from GLMM of foraging scans per day. The reference dominance category is low social rank; the reference reproductive stage is cycling (pre-conception). The grey vertical line represents "no effect". Values to the right of the grey line represent positive effects and values to the left represent negative effects. Significant predictors (p < 0.05) are denoted with asterisks. We present an alternative way to visualise the effect of reproductive stage on foraging activity by plotting the predicted number of resting scans per day for each level of reproductive stage variable (**B**).

*Aim II:* **Investigate gut microbial changes in female capuchins among cycling, pregnant, and nursing states**

Gut microbial community structure remained consistent among reproductive states

Overall, when we examined broad metrics of gut microbial community structure, we found that reproductive state was not a significant predictor of the Chao1 species richness or Shannon alpha diversity (Supplemental Table 3). Rainfall was significantly negatively correlated with Chao1 richness (Incidence Rate Ratio = 0.89, CI = 0.83 – 0.95, p = 0.001), but no other predictors were significant in either model. Reproductive status was not a significant predictor of gut microbial community dissimilarity (DF= 2, F = 1.275, R^2 = 0.008, P = 0.163) and samples from the same

200 reproductive state did not cluster distinctly (Figure 5). Individual identity accounted for a

201 statistically significant degree of dissimililarity among samples (DF = 28, F = 1.278, R^2 = 0.11, P

202 = 0.003), as did diet type and rainfall (Supplemental Table 3).

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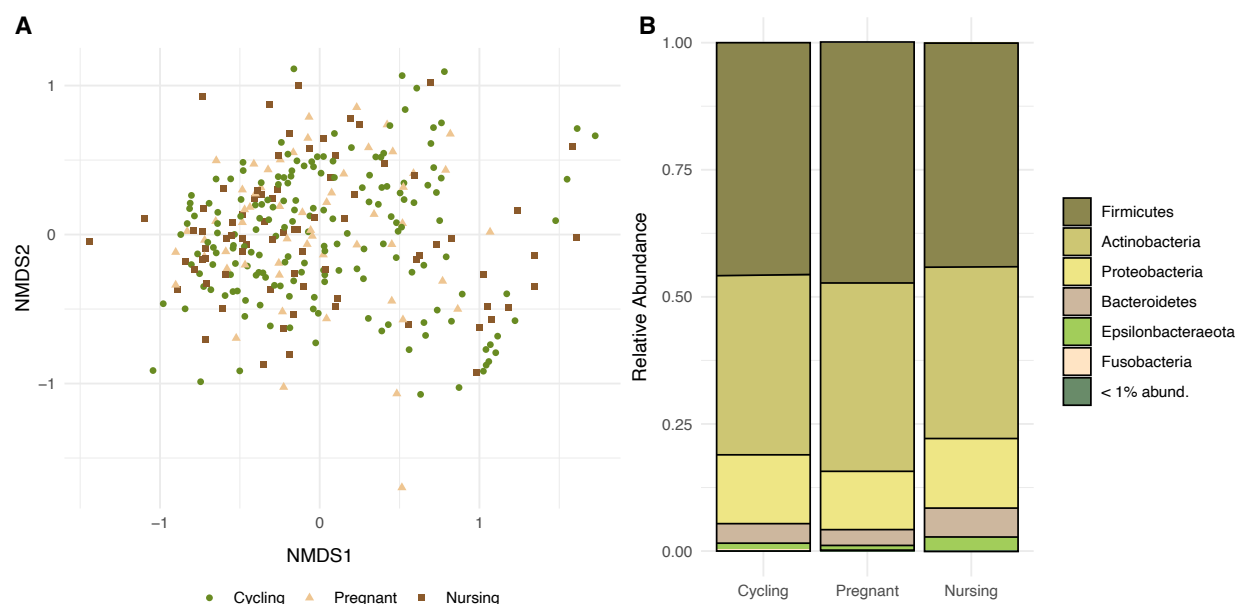
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205 **Figure 5**. For each sample, Bray-Curtis dissimilarity values were computed and ordinated using

206 non-metric multidimensional scaling (NMDS). Samples did not cluster according to reproductive

207 status which was also not a significant predictor of dissimilarity among fecal microbial

208 communities. Relative abundance of phyla were visualized across reproductive statuses. Phyla

209 with relative abundances below 0.01 were grouped in the category "<1% abund." Relative

210 abundances remained generally stable across the reproductive states.

211

212 To investigate other structural changes in the fecal microbial communities among reproductive

213 states, we visualized the relative abundance of phyla across the reproductive cycle, grouping all

214 phyla with relative abundances lower than 1% (Figure 5b). We found that pregnant females

215 exhibited a small but significant increase in Firmicutes taxa compared to cycling females (log2

216 fold change = 0.493, SE = 0.146, P = 0.008). Cycling females exhibited a small but significant

217 increase in the phylum Epsilonbacteraeota (log2 fold change = 1.149, SE = 0.293, P < 0.001).

218 We also examined whether specific genera were differentially abundant among the reproductive

219 states and found that cycling females exhibited a significant increase in taxa from the genus

220 *Helicobacter* compared to nursing females (log2 fold change = 1.226, SE = 0.309, P = 0.01).

Estimated metabolic pathways remain largely stable among reproductive states

Reproductive status was a significant predictor of estimated metabolic pathway dissimilarity, but the effect was small (PERMANOVA; df =2, F Value = 2.5075, R^2 = 0.017, p < 0.001). Nursing females were characterized by a significant increase in pathways related to biotin metabolism (Linear Discriminant Analysis; LDA Score = 3.176, p = 0.006), but otherwise metabolic pathways did not differ substantially between reproductive statuses.

DISCUSSION

We analyzed a robust set of >13,000 individual scans to explore behavioral responses to reproduction and 308 fecal samples to understand gut microbial community dynamics in population of reproductively mature female capuchin monkeys. Our main findings are 1) reproductive state explains some variation in activity budget; in particular, foraging decreases significantly in early nursing compared to cycling, though resting and foraging activity remain otherwise stable across the reproductive cycle; 2) reproductive state explains some variation in gut microbial dissimilarity and relative abundance of putative metabolic pathways; 3) ecological and social variables including maximum temperature, social dominance, and estimated fruit biomass, as well as individual identity are related to activity and the gut microbiota more strongly than reproductive status.

Activity budgets remain largely consistent within and among reproductive states

In our first aim we sought to compare activity budgets of white-faced capuchins among and within cycling, pregnancy, and nursing states at a fine scale. Resting behavior was largely consistent across the reproductive cycle, but the variation observed suggests that resting increases slightly during pregnancy. This pattern fits well with expectations from available data on energy expenditure. While no single stage in the reproductive cycle was stastically significantly different from the other stages in terms of resting, we observed a general pattern that suggests resting increases during pregnancy. Foraging behavior decreased steadily during pregnancy, possibly in connection to resting increases.

252

253   During nursing, resting behavior peaked in early nursing, before decreasing in mid-nursing, and

254   rebounding in late nursing. Foraging behavior decreased steadily into early nursing, where it was

255   significantly lower in early nursing compared to other stages. We recognize that proportion of

256   scans per day spent in foraging states is an imperfect estimator for amount of food consumed.

257   Nevertheless, the statistically significant drop in foraging behavior during early nursing may

258   have energy balance implications for females. Capuchins may alter behavior in other ways to

259   cope with changing energy needs. For example, past research on a small subset of the current

260   study population suggests that lactating females increase feeding rate (McCabe & Fedigan,

261   2007), though we unfortunately lack the required depth of focal data to test this hypothesis in the

262   current data set. There might also be underlying metabolic or other physiological changes such

263   as metabolic shifts associated with energy sparing that we were not able to capture in the present

264   study that help pregnant and lactating females address energy costs. Even though our sample size

265   of 33 females tracked over multiple years and pregnancies is one of the largest available for wild

266   primates, the pattern we observed in the resting behavior may be too subtle to reach significance

267   with present sample sizes.

268

269   While capuchins are generally considered highly flexible and plastic in response to changes in

270   their environments (Fragaszy et al., 2004), it is likely that both resting and foraging behavior is

271   relatively constrained in this population and are influenced by social and environmental factors,

272   limiting the potential for flexibility in this domain in response to reproductive state. When food

273   and water resources change drastically from season to season, capuchins alter their foraging and

274   ranging behaviors to overlap with available food and water (Campos & Fedigan, 2009). We also

275   see changes in thermoregulatory behaviors; capuchins rest more during the hottest parts of the

276   day in the hotter season of the year, and exhibit seasonal behaviors likely linked to

277   thermoregulation and water consumption. It is also possible that female capuchins are

278   constrained in altering activity budget due to the pressures associated with group living. White-

279   faced capuchins form cohesive groups, with the exception of emigrant males dispersing to non-

280   natal groups. Females remain the same social group their entire lives (with the rare exception of

281   group fissioning events), and capuchins forage, rest, and travel in close proximity to one another.

282   Pregnant and lactating females may theoretically benefit from resting for longer periods of the

283   day or foraging for longer periods in a particularly productive food patch; however, if a pregnant

284   or lactating female acts independently of the larger social group, she may be increasing risk of

285   predation or encounters with other social groups. The ability of females to significantly alter

286   resting or foraging may be constrained by the behavioral choices in the rest of the social group.

287   Future studies examining these constraints on activity budget shifts represent an exciting future

288   avenue of behavioral research.

289

290   *Gut microbial communities remain largely stable structurally and functionally throughout*

291   *reproduction*

292

293   We found mixed support for the prediction that females modulate the gut microbiome to increase

294   energy absorption from food during pregnancy and nursing. We did not observe statistically

295   significant changes in alpha diversity in pregnant females, which contrast previous studies in

296   humans that showed a drastic decrease in alpha diversity (Koren et al., 2012). Females in

297   cycling, pregnant, and nursing states clustered separately in a beta diversity plot, but the effect

298   was very small, suggesting alternative drivers of community dissimilarity, including individual

299   variation. Overall, female capuchins did not exhibit large gut microbial structural shifts, but we

300   did find that pregnant females exhibited small but significant increases in Firmicutes taxa. At a

301   broad scale, taxa within Firmicutes break down carbohydrates that endogenous host enzymes are

302   unable to metabolize. The ratio of Firmicutes to Bacteroidetes has previously been suggested as a

303   biomarker for increased metabolic activity in the gut (Turnbaugh et al., 2006); however,

304   contrasting reports of this ratio suggest it might not serve as a universal biomarker for increased

305   energy (Magne et al., 2020). Further, the lack of substantial change in relative abundance among

306   bacterial genera in our samples suggest that, in this population, reproductive state is not a critical

307   driver of gut microbial community composition, at least at a broad scale.

308

309   Though gut microbial communities remained largely stable, nursing capuchins in our population

310   exhibited a significant increase in a biotin metabolism pathway in the gut. Endogenous enzymes

311   as well as gut microbes can metabolize biotin, which is involved in a broad range of metabolic

312   processes related to fat-, carbohydrate-, and amino acid-utilization in mammals. Biotin

313   deficiency and biotin excess during the reproductive has been linked to tetratogenic effects in

13

314 pregnancy in mice and humans (Báez-Saldaña et al., 2009). Studies of humans have repeatedly

315 demonstrated that lactation and pregnancy alter biomarkers of biotin metabolism, and that

316 humans are typically deficient in biotin during pregnancy, though precise requirements of biotin

317 remain unknown (Mock et al., 2002; Perry et al., 2014). If non-human primates also require

318 increased biotin during gestation and lactation, our results suggest that the gut microbiome may

319 play an important role in helping nursing females increase biotin supplementation during fetal

320 growth and infant development.

321

322 Previous studies of non-human primates tend to suggest that the gut microbiome shifts

323 considerably throughout the reproductive cycle (Amato et al., 2014; Mallott et al., 2020; Mallott

324 & Amato, 2018). In a recent examination of white-faced capuchin reproductive microbial

325 ecology, Mallott and Amato (2018) examined how gut microbial communities changed across

326 reproductive states in females ($n_{females} = 5$, $n_{samples} = 39$) sampled across one year in an

327 aseasonally breeding population of white-faced capuchins. The authors found evidence to

328 suggest that the gut microbiome shifts significantly during the reproductive cycle, including

329 differences in relative abundance of Firmicutes (lower in pregnant versus cycling females) and

330 Actinobacteria (higher in pregnant versus lactating females). Further, the authors found that

331 reproductive state was significantly associated with energy and glycan metabolism (Mallott &

332 Amato, 2018). However, this capuchin population lives in a wet aseasonal forest, with little

333 variation in food and water availability throughout the annual cycle (Mallott et al., 2018). The

334 biome where our present study took place is, by contrast, highly seasonal, with distinct shifts in

335 temperature, water availability and fruit and arthropod abundance (Campos et al., 2015;

336 Mosdossy et al., 2015). In the hot, dry season animals contend with harsh drought and high

337 temperature, while in the rainier, cooler season, these forces are less present. We have observed

338 strong effects of seasonality on ranging behavior, activity budget, food choice, and the gut

339 microbiota in this population (Campos et al., 2014; Campos & Fedigan, 2009; Melin et al., 2020;

340 Orkin et al., 2019; Orkin et al., 2019). Extreme seasonality at the present study site and

341 aseasonality at a different site that is home to the same species of capuchins may have critical

342 implications for our understanding of how flexible and plastic this species is across its home

343 range.

344

14

345 Capuchins are generally thought of as one of the most flexible and generalist species of

346 platyrrhine primates that can thrive in a wide variety of habitats and that can consume a vast

347 diversity of food types (Fragaszy et al., 2004; Melin et al., 2020). However, the reality might be

348 a bit more nuanced. Steig Johnson and Kerry Brown (2018) examined niche breadth in

349 Mesoamerican primates using an ecological niche modeling approach, and found that capuchins

350 were considerably constrained by several ecological factors, including precipitation in particular

351 seasons, and seaonality of temperatures. The temperatures and water availability at our study site

352 near the limit of suitable conditions for this species, which may explain the lack of flexibility that

353 we see in behavior and gut microbiota in this population. Understanding how flexibility shifts

354 across a species range and identifying what ecological factors permit or constrain a species'

355 ability to be flexible, is critical to understand not only that species' history, but also how it might

356 fare as ecosystems face anthropogenic and climate-related changes.

357

358 Alternatively, we may be missing the importance of individual variation in response to

359 reproductive states. For example, humans residing in the same population display remarkable

360 differences in response to reproductive demands across our global range; for example, women in

361 the Gambia and Sweden experience high within-group variation in weight gain and energy

362 expenditure throughout pregnancy (Poppitt et al., 1993, 1994) and high inter-individual gut

363 microbiota among members of the same population has been found in humans (Healey et al.,

364 2017; Zhu et al., 2015). We found that individual identity accounted for a significant amount of

365 gut microbial community dissimilarity, which raises exciting questions about individual

366 strategies for coping with reproduction. Further, while activity budgets and amplicon sequencing

367 provide important, thought relatively coarse, data about behavior and gut microbiota

368 respectively, future research on this population of capuchin could incorporate individual focal

369 data and/or shotgun metagenomic sequencing, both of which would provide a more detailed

370 understanding of capuchin reproductive behavioral and microbial ecology.

371

372 How animals respond to the demands of reproduction has important consequences for the

373 viability of offspring, and on a longer term scale, the fitness of a population or species. The

374 intricacies of how animals are able to shift their behavior and how their gut microbial

375 communities may respond to pregnancy and lactation represent a complex but critical area of

15

376 research. For populations living near the ecological limits of their species ranges, it is especially

377 important to understand the extent to which plasticity in behavior and gut microbial communities

378 might influence pregnancy outcomes and multi-generational fitness.

379

380 **MATERIALS AND METHODS**

381

382 *Field site & study population*

383

384 We collected samples and behavioral data at Sector Santa Rosa (SSR), located in the Área de

385 Conservación Guanacaste (ACG), in Guanacaste, Costa Rica (10°53′01′′N 85°46′30′′W).

386 Sector Santa Rosa is a mosaic of forest types, including tropical dry forest and small patches of

387 older growth evergreen forest. The ACG experiences two distinct seasons: a hot, dry period from

388 late November to mid-May and a cooler, rainy period for the remainder of the year, during which

389 almost all of the annual rainfall (900 mm-2400 mm) occurs (Melin et al., 2020). Fruit abundance

390 varies throughout the year and estimates of fruit biomass are calculated monthly (Campos et al.,

391 2015; Orkin et al., 2019).

392

393 The study population of white-faced capuchin monkeys has been continuously monitored non-

394 invasively since 1983. Female capuchins are philopatric and reach reproductive maturity by 6

395 years of age. Births are moderately seasonal at Sector Santa Rosa, with 44% of births occurring

396 between May and July each year (Carnegie et al., 2011). Gestation is 157 +/- 8 days and typical

397 inter-birth intervals are 2.5 years (Melin et al., 2020). Lactation lasts for approximately 12

398 months; in early lactation, infants are almost exclusively dependent on their mothers and are

399 observed nursing frequently (Fragaszy et al., 2004). It should be noted that in other white-faced

400 capuchin populations, the lactation phase can extend to 23 months (Melin et al., 2020). After the

401 first three months of nursing, infants gradually learn to forage independently and incorporate

402 non-milk foods into their diet. By 12 months of age, infants are rarely observed nursing or

403 traveling dorsally on their mothers. Infant capuchins are occasionally observed nursing from

404 adult females that are not their mothers (Sargeant et al., 2016).

405

406   We collected data across 5 years (2014-2018). Non-invasive fecal samples were collected from

407   33 adult females from 4 social groups during multiple sampling bouts in 2014-2016. We

408   collected behavioral data from 33 adult females from 4 social groups during multiple sampling

409   bouts in 2016-2018. One female from the 2014-2016 fecal sampling periods disappeared from

410   the population and was not included in behavioral analysis but was included in gut microbiome

411   analysis. Two females reached sexual maturity during the 2016-2018 period and were included

412   in behavioral analysis but not in gut microbiome analysis. All animals in the study population are

413   habituated to researcher presence and individually identifiable through physical markings on the

414   face and body. In our dataset, 2016 was the only year in which we collected behavioral records

415   and fecal samples simultaneously. Ideally, we would have behavioral records and fecal samples

416   for all 5 years, but this was not possible due to field and laboratory limitations. However, the

417   nearly all individuals (31/33) were present across the entire study period and each dataset

418   (behavioral and fecal) is robust and overlapping.

419

420   During bi-monthly censuses at the field site, reproductive state of each female is recorded.

421   Pregnancies during the study period were determined via protrusion of the abdomen (visible

422   approximately 8 weeks after conception), and after infant were born we estimated conception

423   dates using 157 days as gestation length. At 15 time points throughout the 5-year study, females

424   exhibited protruding abdomens consistent with pregnancy, but then were later observed with flat

425   abdomens. We characterised these instances as pregnancy lsos, though we do not have hormonal

426   data to confirm these pregnancies, which is a limitation associated with this assumption. We

427   determined nursing on an *ad libitum* basis through observations of young monkeys suckling from

428   adult females. Following Bergstrom (2015), we considered females nursing their own infants

429   <12 months of age to be lactating. Juvenile capuchins are occasionally observed suckling after

430   12 months of age, but it is difficult to determine whether milk is transferred. We did not consider

431   cases in which juveniles >12 months were suckling to be indicative of lactation in the adult

432   females from whom they were attempting to nurse. We considered females that were never

433   observed as nursing or pregnant on data collection days as non-pregnant, non-nursing. We

434   grouped all non-pregnant, non-nursing females into the category "cycling" following Bergstrom

435   (Bergstrom, 2015).

436

17

437   Studies of humans and non-human primates suggest that energy requirements change throughout

438   pregnancy and lactation (Emery Thompson, 2013). To examine differences that occur *within*

439   each reproductive state, we subset the reproductive states into stages: Cycling (Pre-conception),

440   Pregnancy Stage 1 (early), Pregnancy Stage 2 (mid), Pregnancy Stage 3 (late), Nursing Stage 1

441   (early), Nursing Stage 2 (mid), Nursing Stage 3 (late), and Cycling (Post-weaning) (Table 1).

442

443   **Table 1**. Pregnancy and nursing were divided into three equal stages. Cycling (Pre-conception)

444   consisted of 60 days prior to a conceptive event, and Cycling (Post-weaning) consisted of 60

445   days post-weaning.

446

Reproductive State	Stage	Length
Cycling (Pre-conception)	--	60-0 days before conception
Pregnancy	early	0-53 days post conception
	mid	54-104 days post conception
	late	105-158 days post conception
Nursing	early	0-121 days postpartum
	mid	122-242 days postpartum
	late	243-365 days postpartum
Cycling (Post-weaning)	--	0-60 days post weaning

447

448   The reproductive state of each of the 33 adult female capuchins is presented in Figure 6.

449   Throughout the 2014-2018 study period, 43 infants were born in the study population.

450   Behavioral data collection periods (2016-2018) included portions of or the full duration of 40 of

451   these pregnancies. Of these 40 infants, 26 infants survived to weaning (365 days), and behavioral

452   data collection included portions of all 40 nursing periods and captured transitions from nursing

453   to non-nursing states.

454
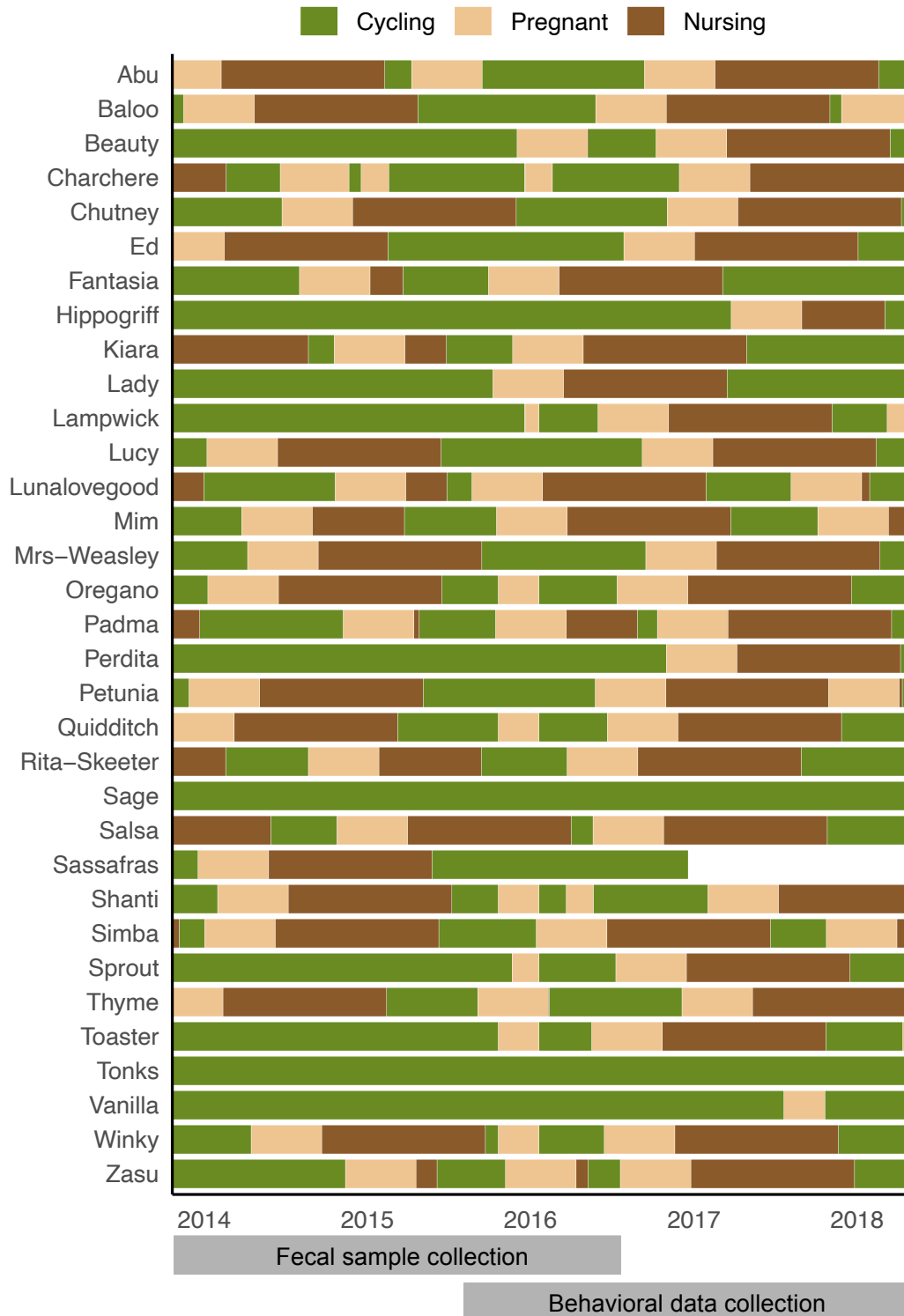
Figure 6. Reproductive status of study individuals observed between April 1, 2014 and June 30, 2018. Fecal samples were collected between April 29, 2014 and September 27, 2016. Behavioral

458   data were collected between April 20, 2016 and June 22, 2018. One individual (Sassafras)

459   disappeared from the population in early 2017. Two individuals (Tonks and Vanilla) reached

460   reproductive maturity during the study, but were never observed to be pregnant. Multiple

461   females were observed to be pregnant via protrusion of the abdomen, but were subsequently

462   observed with no protrusion. In these cases the tan (pregnancy) segments are followed by green

463   (cycling) and not by brown (nursing) segments. Nursing segments that are shorter than 12

464   months represent cases where infants died.

465

466   *Daily individual activity budgets*

467

468   To determine daily activity budgets, we followed each of the 4 social groups from dawn (05:30)

469   until dusk (18:00) for 4-6 days per month. Individual scans were recorded every 30 minutes on

470   the hour and half hour. During a 10-minute period, we recorded the behavioral state of each

471   individual in the group using an established ethogram (Supplemental Table 1). We chose to use

472   scan sampling instead of focal sampling to determine individual activity budgets because it

473   allows for more evenly distributed data across all individuals, season, and time of day (De

474   Ruiter, 1986; Melin et al., 2018). Inter-observer reliability was tested daily for the first 4 weeks

475   of each sampling period, then weekly or biweekly for the remainder of each period. We collected

476   13721 individual scans over the course of 222 contact days.

477

478   *Behavioral models for activity budget analysis*

479

480   We fit two generalized linear mixed models (GLMMs) that included reproductive stage as our

481   predictor of interest. For our Resting Model, number of resting scans per day was our response

482   variable, while for our Foraging Model, number of foraging scans per day was our response

483   variable. In each model, we included monkey dominance category, daily maximum temperature

484   (°C), daily rainfall (cm), and mean monthly estimated fruit biomass (kg/ha) as predictor variables

485   as they may influence activity in this population. Ecological variables (e.g. maximum

486   temperature, rainfall, and fruit biomass) were *z*-transformed (i.e. scaled so that each had a mean

487   of 0 and standard deviation of 1) to stabilize the models. We included individual animal identity

488   as a random effect in all models. Sampling effort (i.e. number of scans per animal per rotation)

489   varied due to changing field conditions and stochastic movement and dispersal of group

490   members. We included a log-transformed offset of total scans per animal per day to account for

491   differences in sampling effort. Because our behavioral data are count data and because

492   behavioral scans occur independently, a Poisson distribution with a logit link was designated in

493   all models.

494

495   We tested whether our alternative models (fixed and random effects) outperformed the null

496   models (random effects only). Likelihood ratio tests were conducted using the R function

497   ANOVA. To test for multicollinearity between ecological variables a generalized linear model

498   (GLM) was created to determine the variance inflation factor (VIF) (Craney & Surles, 2002).

499   These models were identical to the alternative models above but contained only fixed effects.

500   The resulting VIF measures collinearity in fixed effects. Craney and Surles (2002) suggest that

501   appropriate cutoffs for VIF range from 5-10. All ecological variables had VIF indices below 2.0

502   and were kept in all models (Supplemental Table 4).

503

504   We computed incidence rate ratios using the outputs of our GLMMs to examine the effects of

505   each predictor variable. For categorical variables, the incidence rate ratios represent the ratio of

506   the number of scans recorded in one level compared to the number of scans recorded in another

507   level. For variables with multiple levels (e.g. reproductive stage, dominance), a reference level is

508   selected and other levels are compared to the reference level to contextualise the effects of each

509   level on the response variable—in our case, resting scans or foraging scans. We plotted the

510   predicted outcomes for each reproductive stage using the plot_model function in the R package

511   sjPlot (Lüdecke, 2021). Visualising our data this way allowed us to identify patterns of resting

512   behavior or foraging behavior associated with reproductive stage while holding constant the

513   effect of all other predictors in our models.

514

515   *Fecal sampling for gut microbiota analysis*

516

517   We collected fresh fecal samples from study individuals 1-2 times per month within each

518   sampling period in 2014-2016. Once an animal defecated, we immediately collected the feces

519   into a sterile 2mL cryovial using personal protective equipment to minimize human

21

520   contamination of the samples. Fecal samples were visually inspected for dietary components,

521   many of which are identifiable by seed shape or arthropod exoskeletons remnants in the feces.

522   Dietary components were recorded to the most specific taxonomic classification possible.

523   Samples were stored on ice in insulated field packs for a maximum of 5 hours before being

524   transferred to a liquid nitrogen shipper (-90 C) for the remainder of the field season. If a sample

525   was suspected to be contaminated or compromised (e.g. falling on an area where a previous

526   defecation occurred), it was not collected. At the conclusion of each sampling season, samples

527   were shipped to the University of Calgary for processing. Samples were collected with

528   permission from the government of Costa Rica from CONAGEBIO (Approval No. R-025-2014-

529   OT-CONEGABIO) and exported under the Área de Conservación Guanacaste permit (DSVS-

530   029-2014-ACG-PI-060-2014). Samples were imported into Canada with permission from the

531   Canadian Food Inspection Agency (Import Permit: A-2016-03992-4). All data collection

532   complied with Costa Rican law and were approved by University of Calgary Animal Care

533   Committee (#AC15-0161).

534

535   *Laboratory processing*

536

537   Prior to DNA extractions, all laboratory equipment was bleached, autoclaved, and exposed to 60

538   minutes under UV light to minimize laboratory contamination. We randomized the order in

539   which samples were extracted to decrease bias associated with batch effects. Prior to DNA

540   extraction, we split each sample into two tubes, Extraction A and Extraction B to decrease

541   potential bias based on stochastic distribution of microbes within a fecal. During each round of

542   extractions, we extracted a laboratory blank to control for contamination from laboratory

543   surfaces or reagent. The extraction protocol included a bead-beading step to increase DNA yield

544   and specific optimizations are described in Orkin et al. (2019). We purified extracted DNA using

545   an Invitrogen PureLink PCR Purification kit (ThermoFisher Scientific Part No. K310001), after

546   which we combined extractions A and B prior to library preparation. Illumina amplicon

547   sequencing libraries were prepared in for the V4 region of the 16S rRNA gene at the University

548   of Minnesota following Gohl et al. (2016). Libraries were sequenced twice at the University of

549   Calgary to increase reads per sample on an Illumina MiSeq using v2 chemistry.

550

551 *Amplicon data preparation*

552

553 Raw reads were demultiplexed and sequencing barcodes and indices were removed using

554 cutadapt (Martin, 2011). We removed ambiguous base calls using the filterAndTrim function in

555 the R package DADA2, removed locus-specific primers using cutadapt, then determined quality

556 profiles using the plotQualityProfile (Callahan et al., 2016). Poor quality bases were truncated

557 again using the filterAndTrim function. Error rates were learned and dereplication was done

558 using learnErrors and derepFASTQ functions respectively. We merged forward and reverse

559 reads to generate amplicon sequence variants (ASVs). Chimeras were removed using the

560 removeBimeraDenovo function in DADA2, and we assigned taxonomies to ASVs using the

561 silva_nr_v132_train_set.fa file. We extracted and sequenced a series of negative lab controls,

562 which were then used to detect potential contaminants in the program decontam (Davis et al.,

563 2018). Using the function isContaminant, we compared the prevalence of sequences from the

564 negative controls with sequences from our samples. Using a 50% probability threshold, we

565 identified probable contaminants and removed them from our fecal sample sequences. We then

566 removed uncharacterized phyla, chloroplasts, and mitochondrial sequences from the dataset.

567

568 *Gut microbiota community structure*

569

570 To explore shifts in gut microbial community structure throughout the reproductive cycle, we

571 computed Chao species richness and Shannon alpha diversity for each sample. We removed 4

572 samples with Chao1 richness values >400 that were distinctly different that the remaining 304

573 samples, with Chao1 values ranging from 12-385. Because we sampled individuals multiple

574 times, and because sampling effort across individuals was uneven, we fit linear mixed effects

575 models to examine the relationship between reproductive state and richness and diversity

576 metrics. We included individual identity as a random effect in both models and included rainfall

577 and maximum temperature as ecological predictors. We used an alpha of 0.05 as a significance

578 cut-off.

579

580 We then removed extremely low-prevalence phyla for the remainder of analysis and filtered out

581 taxa that were not present in at least 5% of samples. Due to sample size constraints, we were not

582   able to divide fecal samples into subsets within reproductive states and therefore proceeded with

583   the categories cycling, pregnant, and nursing. To explore the relationship between reproductive

584   state and gut microbial community dissimilarity within our sample set, we transformed sample

585   counts to relative abundances and then computed Bray-Curtis dissimilarity values using the

586   ordinate function in phyloseq. We visualized beta diversity using non-metric multidimentional

587   scaling (NMDS). We used the function adonis in the R package vegan to run a PERMANOVA

588   to examine predictors of Bray-Curtis dissimilarities in our dataset (Dixon, 2003). In this

589   PERMANOVA, we included reproductive status as our predictor of interest, as well as

590   individual identity, rainfall, and dietary category based on fecal contents, as we suspected these

591   could be related to microbial community dissimilarity.

592

593   *Differential abundance*

594

595   To examine which, if any, bacterial taxa were differentially abundant among reproductive states,

596   we agglomerated samples at the genus level, then used the R package DESeq2 to compute

597   variance stabilized counts (Love et al., 2014). We then used Wald tests to determine the log2

598   fold differences among the reproductive states and used adjusted *P* values (alpha = 0.01) to

599   account for multiple tests. We conducted pairwise comparisons between cycling and pregnant

600   females, pregnant and nursing females, and nursing and cycling females to examine how these

601   transitions might be related to gut microbial community structure. We repeated this analysis for

602   all bacterial phyla in our dataset to examine courser scale shifts in fecal microbial community

603   structure among reproductive states.

604

605   *Estimated metabolic pathways*

606

607   We used the package PICRUSt2 to estimate metabolic pathways present in our samples using

608   KEGG orthologs (Douglas et al., 2020). We tested for significant dissimilarity in estimated

609   metabolic pathways among the reproductive states using a PERMANOVA including individual

610   identity as a control. We then used the linear discriminant analysis (LDA) effect size method

611   using the LEfSe package (Segata et al., 2011), which identifies the functional metabolic

612   pathways likely to explain differences between reproductive states and stages in our data. We

24

613   used a logarithmic LDA score of 2 as a cut off for discrimant features, and individual identity

614   was included as a predictor to account for individual variation. All code used for analysis in this

615   study is available at

616   https://github.com/webbshasta/CapuchinReproductionBehaviourMicrobiome.

617

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633   **COMPETING INTERESTS**

634   The authors declare no competing interests.

635   **REFERENCES**

636

637   Altmann, J. (2001). *Baboon Mothers and Infants*. University of Chicago Press.

638   Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., Wilson, B.

639   A., Nelson, K. E., White, B. A., & Garber, P. A. (2014). The role of gut microbes in

25

640   satisfying the nutritional demands of adult and juvenile wild, black howler monkeys

641   (*Alouatta pigra*). *American Journal of Physical Anthropology*, *155*(4), 652–664.

642   Báez-Saldaña, A., Camacho-Arroyo, I., Espinosa-Aguirre, J. J., Neri-Gómez, T., Rojas-Ochoa,

643   A., Guerra-Araiza, C., Larrieta, E., Vital, P., Díaz, G., Chavira, R., & Fernandez-Mejia,

644   C. (2009). Biotin deficiency and biotin excess: Effects on the female reproductive

645   system. *Steroids*, *74*(10), 863–869. https://doi.org/10.1016/j.steroids.2009.06.004

646   Bergstrom, M. L. (2015). *Seasonal Effects on the Nutrition and Energetic Condition of Female*

647   *White-Faced Capuchin Monkeys* [Doctoral dissertation]. University of Calgary.

648   Bowen, W. D., Ellis, S. L., Iverson, S. J., & Boness, D. J. (2001). Maternal effects on offspring

649   growth rate and weaning mass in harbour seals. *Canadian Journal of Zoology*, *79*(6),

650   1088–1101.

651   Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P.

652   (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature*

653   *Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

654   Campos, F. A., Bergstrom, M. L., Childers, A., Hogan, J. D., Jack, K. M., Melin, A. D.,

655   Mosdossy, K. N., Myers, M. S., Parr, N. A., Sargeant, E., Schoof, V. A. M., & Fedigan,

656   L. M. (2014). Drivers of home range characteristics across spatiotemporal scales in a

657   Neotropical primate, *Cebus capucinus*. *Animal Behaviour*, *91*, 93–109.

658   https://doi.org/10.1016/j.anbehav.2014.03.007

659   Campos, F. A., & Fedigan, L. M. (2009). Behavioral adaptations to heat stress and water scarcity

660   in white-faced capuchins (*Cebus capucinus*) in Santa Rosa National Park, Costa Rica.

661   *American Journal of Physical Anthropology*, *138*(1), 101–111.

662 Campos, F. A., Jack, K. M., & Fedigan, L. M. (2015). Climate oscillations and conservation

663   measures regulate white-faced capuchin population growth and demography in a

664   regenerating tropical dry forest in Costa Rica. *Biological Conservation*, *186*, 204–213.

665 Carnegie, S., Fedigan, L., & Melin, A. (2011). Reproductive Seasonality in Female Capuchins

666   (*Cebus capucinus*) in Santa Rosa (Area de Conservación Guanacaste), Costa Rica.

667   *International Journal of Primatology*, *32*(5), 1076–1090. afh.

668   https://doi.org/10.1007/s10764-011-9523-x

669 Clutton-Brock, T. H., Albon, S. D., & Guinness, F. E. (1989). Fitness costs of gestation and

670   lactation in wild mammals. *Nature*, *337*(6204), 260–262.

671   https://doi.org/10.1038/337260a0

672 Craney, T. A., & Surles, J. G. (2002). Model-Dependent Variance Inflation Factor Cutoff

673   Values. *Quality Engineering*, *14*(3), 391–403. https://doi.org/10.1081/QEN-120001878

674 Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple

675   statistical identification and removal of contaminant sequences in marker-gene and

676   metagenomics data. *Microbiome*, *6*(1), 226. https://doi.org/10.1186/s40168-018-0605-2

677 De Ruiter, J. R. (1986). The influence of group size on predator scanning and foraging behaviour

678   of wedgecapped capuchin monkeys (*Cebus olivaceus*). *Behaviour*, *98*(1), 240–258.

679 Deschner, T., Kratzsch, J., & Hohmann, G. (2008). Urinary C-peptide as a method for

680   monitoring body mass changes in captive bonobos (*Pan paniscus*). *Hormones and*

681   *Behavior*, *54*(5), 620–626. https://doi.org/10.1016/j.yhbeh.2008.06.005

682 Dewey, K. G. (1997). Energy and Protein Requirements During Lactation. *Annual Review of*

683   *Nutrition*, *17*(1), 19–36. https://doi.org/10.1146/annurev.nutr.17.1.19

684   DiGiulio, D. B., Callahan, B. J., McMurdie, P. J., Costello, E. K., Lyell, D. J., Robaczewska, A.,

685   Sun, C. L., Goltsman, D. S. A., Wong, R. J., Shaw, G., Stevenson, D. K., Holmes, S. P.,

686   & Relman, D. A. (2015). Temporal and spatial variation of the human microbiota during

687   pregnancy. *Proceedings of the National Academy of Sciences*, *112*(35), 11060–11065.

688   https://doi.org/10.1073/pnas.1502875112

689   Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of*

690   *Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

691   Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M.,

692   Huttenhower, C., & Langille, M. G. I. (2020). PICRUSt2 for prediction of metagenome

693   functions. *Nature Biotechnology*, *38*(6), 685–688. https://doi.org/10.1038/s41587-020-

694   0548-6

695   Dufour, D. l., & Sauther, M. l. (2002). Comparative and evolutionary dimensions of the

696   energetics of human pregnancy and lactation. *American Journal of Human Biology*,

697   *14*(5), 584–602. https://doi.org/10.1002/ajhb.10071

698   Edwards, S. M., Cunningham, S. A., Dunlop, A. L., & Corwin, E. J. (2017). The Maternal Gut

699   Microbiome during Pregnancy. *MCN. The American Journal of Maternal Child Nursing*,

700   *42*(6), 310–317. https://doi.org/10.1097/NMC.0000000000000372

701   Ellison, P. T. (2003). Energetics and reproductive effort. *American Journal of Human Biology*,

702   *15*(3), 342–351. https://doi.org/10.1002/ajhb.10152

703   Emery Thompson, M. (2013). Comparative reproductive energetics of human and nonhuman

704   primates. *Annual Review of Anthropology*, *42*, 287–304.

705   Fontaine, E. (2012). Food Intake and Nutrition During Pregnancy, Lactation and Weaning in the

706   Dam and Offspring. *Reproduction in Domestic Animals*, *47*(s6), 326–330.

707   https://doi.org/10.1111/rda.12102

708   Fragaszy, D. M., Visalberghi, E., & Fedigan, L. M. (2004). *The Complete Capuchin: The*

709   *Biology of the Genus Cebus*. Cambridge University Press.

710   Gittleman, J. L., & Thompson, S. D. (1988). Energy Allocation in Mammalian Reproduction.

711   *Integrative and Comparative Biology*, *28*(3), 863–875.

712   https://doi.org/10.1093/icb/28.3.863

713   Gohl, D. M., Vangay, P., Garbe, J., MacLean, A., Hauge, A., Becker, A., Gould, T. J., Clayton,

714   J. B., Johnson, T. J., Hunter, R., Knights, D., & Beckman, K. B. (2016). Systematic

715   improvement of amplicon marker gene methods for increased accuracy in microbiome

716   studies. *Nature Biotechnology*, *34*(9), 942–949. https://doi.org/10.1038/nbt.3601

717   Harrison, M. J. (1983). Age and sex differences in the diet and feeding strategies of the green

718   monkey, Cercopithecus sabaeus. *Animal Behaviour*, *31*(4), 969–977.

719   Healey, G. R., Murphy, R., Brough, L., Butts, C. A., & Coad, J. (2017). Interindividual

720   variability in gut microbiota and host response to dietary interventions. *Nutrition*

721   *Reviews*, *75*(12), 1059–1080. https://doi.org/10.1093/nutrit/nux062

722   Johnson, S. E., & Brown, K. A. (2018). The Specialist Capuchin? Using Ecological Niche

723   Models to Compare Niche Breadth in Mesoamerican Primates. In U. Kalbitzer & K. M.

724   Jack (Eds.), *Primate Life Histories, Sex Roles, and Adaptability: Essays in Honour of*

725   *Linda M. Fedigan* (pp. 311–329). Springer International Publishing.

726   https://doi.org/10.1007/978-3-319-98285-4_15

727   Jost, T., Lacroix, C., Braegger, C., & Chassard, C. (2013). Stability of the Maternal Gut

728   Microbiota During Late Pregnancy and Early Lactation. *Current Microbiology*, *68*(4),

729   419–427. https://doi.org/10.1007/s00284-013-0491-6

730   Key, C., & Ross, C. (1999). Sex differences in energy expenditure in non–human primates.

731   *Proceedings of the Royal Society of London B: Biological Sciences*, *266*(1437), 2479–

732   2485. https://doi.org/10.1098/rspb.1999.0949

733   Koren, O., Goodrich, J. K., Cullender, T. C., Spor, A., Laitinen, K., Kling Bäckhed, H.,

734   Gonzalez, A., Werner, J. J., Angenent, L. T., Knight, R., Bäckhed, F., Isolauri, E.,

735   Salminen, S., & Ley, R. E. (2012). Host Remodeling of the Gut Microbiome and

736   Metabolic Changes during Pregnancy. *Cell*, *150*(3), 470–480.

737   https://doi.org/10.1016/j.cell.2012.07.008

738   Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and

739   dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550.

740   https://doi.org/10.1186/s13059-014-0550-8

741   Lunn, P. G., Austin, S., Prentice, A. M., & Whitehead, R. G. (1984). The effect of improved

742   nutrition on plasma prolactin concentrations and postpartum infertility in lactating

743   Gambian women. *The American Journal of Clinical Nutrition*, *39*(2), 227–235.

744   https://doi.org/10.1093/ajcn/39.2.227

745   Magne, F., Gotteland, M., Gauthier, L., Zazueta, A., Pesoa, S., Navarrete, P., & Balamurugan, R.

746   (2020). The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in

747   Obese Patients? *Nutrients*, *12*(5), 1474. https://doi.org/10.3390/nu12051474

748   Mallott, E. K., & Amato, K. R. (2018). The microbial reproductive ecology of white-faced

749   capuchins (Cebus capucinus). *American Journal of Primatology*, *80*(8), e22896.

750   https://doi.org/10.1002/ajp.22896

751   Mallott, E. K., Amato, K. R., Garber, P. A., & Malhi, R. S. (2018). Influence of fruit and

752   invertebrate consumption on the gut microbiota of wild white-faced capuchins (*Cebus*

753   *capucinus*). *American Journal of Physical Anthropology*, *165*(3), 576–588.

754   https://doi.org/10.1002/ajpa.23395

755   Mallott, E. K., Borries, C., Koenig, A., Amato, K. R., & Lu, A. (2020). Reproductive hormones

756   mediate changes in the gut microbiome during pregnancy and lactation in Phayre's leaf

757   monkeys. *Scientific Reports*, *10*(1), 9961. https://doi.org/10.1038/s41598-020-66865-2

758   Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.

759   *EMBnet.Journal*, *17*(1), 10–12. https://doi.org/10.14806/ej.17.1.200

760   McCabe, G. M., & Fedigan, L. M. (2007). Effects of Reproductive Status on Energy Intake,

761   Ingestion Rates, and Dietary Composition of Female *Cebus capucinus* at Santa Rosa,

762   Costa Rica. *International Journal of Primatology*, *28*(4), 837–851. afh.

763   https://doi.org/10.1007/s10764-007-9159-z

764   Melin, A. D., Hogan, J. D., Campos, F. A., Wikberg, E., King-Bailey, G., Webb, S., Kalbitzer,

765   U., Asensio, N., Murillo-Chacon, E., Hernandez, S. C., Chavarria, A. G., Schaffner, C.

766   M., Kawamura, S., Aureli, F., Fedigan, L., & Jack, K. M. (2020). Primate life history,

767   social dynamics, ecology, and conservation: Contributions from long-term research in

768   Área de Conservación Guanacaste, Costa Rica. *Biotropica*, *52*(6), 1041–1064.

769   https://doi.org/10.1111/btp.12867

770 Melin, A. D., Webb, S. E., Williamson, R. E., & Chiou, K. L. (2018). Data Collection in Field

771   Primatology: A Renewed Look at Measuring Foraging Behaviour. In U. Kalbitzer & K.

772   M. Jack (Eds.), *Primate Life Histories, Sex Roles, and Adaptability: Essays in Honour of*

773   *Linda M. Fedigan* (pp. 161–192). Springer International Publishing.

774   https://doi.org/10.1007/978-3-319-98285-4_9

775 Mock, D. M., Quirk, J. G., & Mock, N. I. (2002). Marginal biotin deficiency during normal

776   pregnancy. *The American Journal of Clinical Nutrition*, *75*(2), 295–299.

777   https://doi.org/10.1093/ajcn/75.2.295

778 Mosdossy, K. N., Melin, A. D., & Fedigan, L. M. (2015). Quantifying seasonal fallback on

779   invertebrates, pith, and bromeliad leaves by white-faced capuchin monkeys (*Cebus*

780   *capucinus*) in a tropical dry forest. *American Journal of Physical Anthropology*, *158*(1),

781   67–77.

782 National Research Council, C. on A. N. (2003). Protein. In *Read "Nutrient Requirements of*

783   *Nonhuman Primates: Second Revised Edition" at NAP.edu* (2nd ed., pp. 75–81). National

784   Academies Press. https://doi.org/10.17226/9826

785 Orkin, J. D., Campos, F. A., Myers, M. S., Hernandez, S. E. C., Guadamuz, A., & Melin, A. D.

786   (2019). Seasonality of the gut microbiota of free-ranging white-faced capuchins in a

787   tropical dry forest. *The ISME Journal*, *13*(1), 183. https://doi.org/10.1038/s41396-018-

788   0256-0

789 Orkin, J. D., Webb, S. E., & Melin, A. D. (2019). Small to modest impact of social group on the

790   gut microbiome of wild Costa Rican capuchins in a seasonal forest. *American Journal of*

791   *Primatology*, *81*(11), e22985. https://doi.org/10.1002/ajp.22985

792   Perry, C. A., West, A. A., Gayle, A., Lucas, L. K., Yan, J., Jiang, X., Malysheva, O., & Caudill,

793   M. A. (2014). Pregnancy and Lactation Alter Biomarkers of Biotin Metabolism in

794   Women Consuming a Controlled Diet. *The Journal of Nutrition*, *144*(12), 1977–1984.

795   https://doi.org/10.3945/jn.114.194472

796   Poppitt, S. D., Prentice, A. M., Goldberg, G. R., & Whitehead, R. G. (1994). Energy-sparing

797   strategies to protect human fetal growth. *American Journal of Obstetrics and*

798   *Gynecology*, *171*(1), 118–125.

799   Poppitt, S. D., Prentice, A. M., Jéquier, E., Schutz, Y., & Whitehead, R. G. (1993). Evidence of

800   energy sparing in Gambian women during pregnancy: A longitudinal study using whole-

801   body calorimetry. *The American Journal of Clinical Nutrition*, *57*(3), 353–364.

802   Sargeant, E. J., Wikberg, E. C., Kawamura, S., Jack, K. M., & Fedigan, L. M. (2016). Paternal

803   kin recognition and infant care in white-faced capuchins (*Cebus capucinus*). *American*

804   *Journal of Primatology*, *78*(6), 659–668. https://doi.org/10.1002/ajp.22530

805   Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C.

806   (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*(6),

807   R60. https://doi.org/10.1186/gb-2011-12-6-r60

808   Serio-Silva, J. C., Hernández-Salazar, L. T., & Rico-Gray, V. (1999). Nutritional composition of

809   the diet of *Alouatta palliata mexicana* females in different reproductive states. *Zoo*

810   *Biology*, *18*(6), 507–513. https://doi.org/10.1002/(SICI)1098-2361(1999)18:6<507::AID-

811   ZOO5>3.0.CO;2-R

812   Smid, M. C., Ricks, N. M., Panzer, A., Mccoy, A. N., Azcarate-Peril, M. A., Keku, T. O., &

813   Boggess, K. A. (2018). Maternal gut microbiome biodiversity in pregnancy. *American*

814   *Journal of Perinatology*, *35*(1), 24–30. https://doi.org/10.1055/s-0037-1604412

815   Sun, B., Xu, X., Xia, Y., Cheng, Y., Mao, S., Xiang, X., Xia, D., Wang, X., & Li, J. (2021).

816   Variation of Gut Microbiome in Free-Ranging Female Tibetan Macaques (*Macaca*

817   *thibetana*) across Different Reproductive States. *Animals*, *11*(1), 39.

818   https://doi.org/10.3390/ani11010039

819   Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I.

820   (2006). An obesity-associated gut microbiome with increased capacity for energy harvest.

821   *Nature*, *444*(7122), 1027–1131. https://doi.org/10.1038/nature05414

822   Vasey, N. (2005). Activity budgets and activity rhythms in red ruffed lemurs (*Varecia rubra*) on

823   the Masoala Peninsula, Madagascar: Seasonality and reproductive energetics. *American*

824   *Journal of Primatology*, *66*(1), 23–44. https://doi.org/10.1002/ajp.20126

825   Villar, J., Cogswell, M., Kestler, E., Castillo, P., Menendez, R., & Repke, J. T. (1992). Effect of

826   fat and fat-free mass deposition during pregnancy on birth weight. *American Journal of*

827   *Obstetrics and Gynecology*, *167*(5), 1344–1352. https://doi.org/10.1016/S0002-

828   9378(11)91714-1

829   Zhu, A., Sunagawa, S., Mende, D. R., & Bork, P. (2015). Inter-individual differences in the gene

830   content of human gut bacterial species. *Genome Biology*, *16*(1), 82.

831   https://doi.org/10.1186/s13059-015-0646-9

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Supplemental Table 1. Ethogram of behaviors for white-faced capuchin monkeys at Sector Santa Rosa, Costa Rica

Type of Behavior	Specific Behavior	Code	Description
Foraging	Forage: Insect (Extractive)	EFI	Tearing branches, ripping bark
	Forage: Fruit (Extractive)	EFF	Pounding, scrubbing, or breaking open fruits
	Forage: Flower	FFL	Feeding on flowers
	Forage: Fruit	FFR	Feeding on fruit
	Forage: Insect	FIN	Feeding on insects
	Forage: Other	FOT	Bromeliad leaves, pith, vertebrates
	Forage: Visually	VFO	Actively looking for food, including gleaning insects while moving
	Forage: Out of sight	FOS	Monkey is foraging but mouth and/or forelimbs are not visible
Resting	Rest (Solitary)	RES	Lying alone, not moving
	Rest (Social)	SRE	Not moving, lying down
Travel	Travel	TRA	Travel; moving very rapidly, not pausing for foraging or socializing
Social Affiliation	Social (Active)	SAC	Monkeys are affiliative; allogrooming
Social Aggression	Social (Aggressive)	SAG	Chasing, biting conspecifics
Other	Vigilant	VIG	Scanning intently at a long range (not for food)
	Drink	DRI	Drink
	Excretion	EXC	Excretion of feces, urine, or vomit
	Self-Directed	SDI	Auto groom
	Play	PLA	Play: biting, chasing, hitting, bouncing, pushing, pulling, etc.
	Other	OTH	Inter-group encounter, mobbing predator, sexual behaviour

Supplemental Table 2. Generalized linear mixed models for resting and foraging behaviours. Model results and incidence rate ratios were computed for each prediction.

Prediction	Generalized Linear Mixed Model	Results							
		Predictor	**Estimate**	**Std. Error**	**Z-Value**	**P-Value**	**Incidence Rate Ratios**	**Confidence Interval**	**P-Value**
Females in periods of high energy demand (i.e., pregnancy, nursing) will rest more than females in periods of lower energy demand (i.e., cycling).	TotalRestingScans ~ DominanceCat + RepStateStage + Rainfall_cm + TempMax + MonthlyFruitBiomass + offset(log(TotalScans)) + (1 | Animal), data = dfzGrouped, family = poisson(link = "log"))	(Intercept)	-1.2988	0.08671	-14.98	<2e-16	0.27	0.23 – 0.32	<0.001
		DominanceCatmid	-0.04233	0.06849	-0.618	0.5365	0.96	0.84 – 1.10	0.537
		DominanceCathigh	**-0.13081**	**0.06053**	**-2.161**	**0.0307**	**0.88**	**0.78 – 0.99**	**0.031**
		RepStateStagePregnancyStage1	-0.13774	0.10057	-1.37	0.1708	0.87	0.72 – 1.06	0.171
		RepStateStagePregnancyStage2	-0.04896	0.10391	-0.471	0.6375	0.95	0.78 – 1.17	0.638
		RepStateStagePregnancyStage3	0.08571	0.09624	0.891	0.3732	1.09	0.90 – 1.32	0.373
		RepStateStageNursingStage1	0.11913	0.08252	1.444	0.1488	1.13	0.96 – 1.32	0.149
		RepStateStageNursingStage2	-0.09301	0.10209	-0.911	0.3623	0.91	0.75 – 1.11	0.362
		RepStateStageNursingStage3	0.0879	0.10163	0.865	0.3871	1.09	0.89 – 1.33	0.387
		RepStateStageCycling_PostWeaning	0.11204	0.10777	1.04	0.2985	1.12	0.91 – 1.38	0.299
		Rainfall_cm	-0.04054	0.02174	-1.865	0.0622	0.96	0.92 – 1.00	0.062
		TempMax	**0.22587**	**0.02182**	**10.352**	**<2e-16**	**1.25**	**1.20 – 1.31**	**<0.001**
		MonthlyFruitBiomass	-0.01562	0.02175	-0.718	0.4726	0.98	0.94 – 1.03	0.473
Females in periods of high energy demand (i.e., pregnancy, nursing) will forage more than females in periods of lower energy demand (i.e., cycling).	TotalForagingScans ~ DominanceCat + RepStateStage + Rainfall_cm + TempMax + MonthlyFruitBiomass + offset(log(TotalScans)) + (1 | Group/Animal), data = dfzGrouped, family = poisson(link = "log"))	**Predictor**	**Estimate**	**Std. Error**	**Z-Value**	**P-Value**	**Incidence Rate Ratios**	**Confidence Interval**	**P-Value**
		(Intercept)	-0.54966	0.060256	-9.122	<2e-16	0.58	0.51 – 0.65	**<0.001**
		DominanceCatmid	0.016859	0.042153	0.4	0.6892	1.02	0.94 – 1.10	0.689
		DominanceCathigh	-0.030515	0.031026	-0.984	0.3253	0.97	0.91 – 1.03	0.325
		RepStateStagePregnancyStage1	0.053213	0.061225	0.869	0.3848	1.05	0.94 – 1.19	0.385
		RepStateStagePregnancyStage2	-0.003497	0.068042	-0.051	0.959	1	0.87 – 1.14	0.959
		RepStateStagePregnancyStage3	-0.02176	0.062772	-0.347	0.7289	0.98	0.87 – 1.11	0.729
		RepStateStageNursingStage1	**-0.132814**	**0.053359**	**-2.489**	**0.0128**	**0.88**	**0.79 – 0.97**	**0.013**
		RepStateStageNursingStage2	-0.043159	0.061833	-0.698	0.4852	0.96	0.85 – 1.08	0.485
		RepStateStageNursingStage3	-0.123121	0.067554	-1.823	0.0684	0.88	0.77 – 1.01	0.068
		RepStateStageCycling_PostWeaning	-0.100933	0.072291	-1.396	0.1627	0.9	0.78 – 1.04	0.163
		Rainfall_cm	**0.026021**	**0.013125**	**1.983**	**0.0474**	**1.03**	**1.00 – 1.05**	**0.047**
		TempMax	**-0.139173**	**0.016169**	**-8.608**	**<2e-16**	**0.87**	**0.84 – 0.90**	**<0.001**
		MonthlyFruitBiomass	**0.038289**	**0.016033**	**2.388**	**0.0169**	**1.04**	**1.01 – 1.07**	**0.017**

Supplemental Table 4. Linear mixed model outputs for richness and alpha diversity among fecal samples across reproductive states and PERMANOVA for Bray-Curtis dissimilarity among fecal samples.

Study Component	Model Description	Model	Results							
Chao1 richness among reproductive states	Generalized linear mixed model with negative binomal distribution	chao1~ ReproductiveStatus + scale(Rainfall) + scale(TemperatureMax) + (1|INDIVIDUAL), data=metadataFilt)	**Predictor**	**Estimate**	**Std. Error**	**Z-Value**	**P-Value**	**Incidence Rate Ratios**	**Confidence Interval**	**P-Value**
			(Intercept)	4.76545	0.05274	90.356	<0.0001	117.38	105.86 – 130.17	<0.001
			Nursing	-0.04053	0.09002	-0.45	0.65256	0.96	0.80 – 1.15	0.653
			Pregnant	-0.13895	0.09505	-1.462	0.14377	0.87	0.72 – 1.05	0.144
			scale(Rainfall)	**-0.11816**	**0.03632**	**-3.253**	**0.00114**	**0.89**	**0.83 – 0.95**	**0.001**
			scale(TemperatureMax)	0.01115	0.03721	0.3	0.76439	1.01	0.94 – 1.09	0.764
Shannon alpha diversity among reproductive states	Linear mixed model with Gaussian distribution	alphadiv~ ReproductiveStatus + scale(TemperatureMax) + scale(Rainfall) + (1|INDIVIDUAL), data=metadataFilt)	**Predictor**	**Estimate**	**Std. Error**	**T-Value**	**P-Value**	**Incidence Rate Ratios**	**Confidence Interval**	**P-Value**
			(Intercept)	2.64774	0.0438	60.445	--	--	2.56 – 2.73	<0.001
			Nursing	0.09473	0.07983	1.187	--	--	-0.06 – 0.25	0.235
			Pregnant	0.08834	0.08754	1.009	--	--	-0.08 – 0.26	0.313
			scale(TemperatureMax)	**0.07061**	**0.03513**	**2.01**	**--**	**--**	**0.00 – 0.14**	**0.044**
			scale(Rainfall)	-0.05154	0.03443	-1.497	--	--	-0.12 – 0.02	0.134
Bray-Curtis dissimilarity among reproductive states	PERMANOVA using adonis function R package vegan	distance(psState_filt, method="bray") ~ ReproductiveStatus + INDIVIDUAL + scale(Rainfall)	**Predictor**	**Df**	**Sums of Squares**	**Mean Squares**	**F-Value**	**R²**	**P-Value**	**--**
			Reproductive Status	2	0.614	0.30706	1.239	0.00789	0.186	
			Individual	**28**	**8.618**	**0.30779**	**1.242**	**0.11071**	**0.005**	**--**
			scale(Rainfall)	**1**	**0.711**	**0.71059**	**2.8674**	**0.00913**	**0.003**	**--**

Supplemental Table 4. Generalized linear models to test variance inflation factor for ecological variables.

Generalized Linear Model		GVIF	Df	GVIF^(1/(2*Df))
Variance inflation factor test for ecological variables in Resting Model	glm(TotalRestingScans ~ DominanceCat + RepStateStage + Rainfall_cm + TempMax + MonthlyFruitBiomass + offset(log(TotalScans)), data = dfzGrouped, family = "poisson")			
	DominanceCat	1.080238	2	1.019483
	RepStateStage	1.41904	7	1.025314
	Rainfall_cm	1.197425	1	1.094269
	TempMax	1.280846	1	1.131745
	MonthlyFruitBiomass	1.198814	1	1.094904
Generalized Linear Model		**GVIF**	**Df**	**GVIF^(1/(2*Df))**
Variance inflation factor test for ecological variables in Foraging Model	glm(TotalForagingScans ~ DominanceCat + RepStateStage + Rainfall_cm + TempMax + MonthlyFruitBiomass + offset(log(TotalScans)), data = dfzGrouped, family = "poisson")			
	DominanceCat	1.080238	2	1.019483
	RepStateStage	1.41904	7	1.025314
	Rainfall_cm	1.197425	1	1.094269
	TempMax	1.280846	1	1.131745
	MonthlyFruitBiomass	1.198814	1	1.094904