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1 **Do sexual calls in female black capuchin monkeys (*Sapajus nigritus*) vary with**
2 **fertility? An acoustic analysis**

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23 **Running title:** Sexual calls and fertility in female black capuchin monkeys (*Sapajus nigritus*)

24 **Abstract**

25 Females across a range of animal taxa produce vocalizations and signals uniquely associated
26 with periods of mating. While such signals may ultimately function to increase female
27 attractivity to males, conflicting findings challenge the extent to which these signals co-vary in
28 accordance with the probability of conception. Female black capuchin monkeys (*Sapajus*
29 *nigrinus*) display an elaborate repertoire of both vocal and visual components as part of their
30 socio-sexual behavior, and previous analyses have shown that the rates of production of visual,
31 but not vocal, components provide graded information on female ovulation. It remains possible,
32 however, that the acoustic parameters of these sexual calls, rather than their rate of productions,
33 co-vary with female fertility. To test this, we analyzed structural and temporal call parameters
34 from estrous calls and post-copulatory calls recorded over five consecutive mating seasons in 12
35 sexually mature females at Iguazú National Park, Argentina. Calls given during the fertile phase
36 of the female ovarian cycle were compared with those given during the non-fertile phase, as
37 determined by profiles of female reproductive hormones. Similarly, within the fertile phase, we
38 tested whether temporal or spectral acoustic parameters of calls gradually change with the
39 approach of ovulation. We did not find any significant relationship between call parameters and
40 the two measures of female fertility in either female estrous calls or post-copulatory calls.
41 However, some differences between pre- and post-copulatory calls were apparent. Overall, our
42 results indicate that sexual calls in black capuchin females do not provide precise information
43 about the timing of ovulation, but may allow listeners to make probabilistic inferences about
44 whether copulations have taken place. This, combined with previous findings, suggests that
45 females in our study may use signals in different modalities to convey information about their
46 fertility and sexual behavior with varying degrees of precision.

47

48 **Keywords:** Bioacoustics, vocal communication, sexual signals, female reproductive hormones,

49 *Sapajus nigritus*.

50 **Introduction**

51 Primate females, in contrast to most mammals, have developed a large variety of
52 behavioral and morphological signals that are produced in association with increased sexual
53 activity (Dixson, 2012). Many of these are assumed to be sexually selected traits, and appear to
54 function within the contexts of inter-sexual conflict and mate choice (Andersson, 1994). Female
55 sexual signals are especially common in catarrhines, particularly in those species characterized
56 by multimale-multifemale social organization and promiscuous mating systems (Dixson, 2012).
57 In some species, females produce multiple sexual signals in different modalities (e.g., production
58 of both visual morphological signals and acoustic vocal signals: Nunn, 1999; Pradhan,
59 Engelhardt, van Schaik, & Maestriperi, 2006). In such cases, it is necessary to conduct detailed
60 analysis of all signals in order to understand whether the signals potentially provide receivers
61 with redundant or distinct information (Liebal, Waller, Slocombe, & Burrows, 2013; Partan &
62 Marler, 1999), and to better understand female mating strategies (see Kappeler & van Schaik,
63 2004).

64 Previous studies on sexual signals in female primates have paid particular attention to the
65 extent to which they have the potential to inform receivers about the female's fertility state
66 (Brauch et al., 2007; Deschner, Heistermann, Hodges, & Boesch, 2004; Engelhardt, Hodges,
67 Niemitz, & Heistermann, 2005). While particular attention focused on exaggerated sexual
68 swellings in this regard (Brauch et al., 2007; Engelhardt et al., 2005), it has been suggested that
69 female sexual calls, in most studies referred to as copulation calls, may also provide information
70 regarding the timing of ovulation (e.g., Aujard, Heistermann, Thierry, & Hodges, 1998;
71 Pfefferle, Brauch, Heistermann, Hodges, & Fischer, 2008). However, there is considerable
72 variation across primate species in the extent and the precision to which short-term hormonal

73 changes in female ovarian cycles associate with changes in both the production and acoustic
74 structure of sexual calls (Aujard et al., 1998; Pfefferle, Heistermann, Pirow, Hodges, & Fischer,
75 2011). In human females, for instance, voice characteristics such as frequency and amplitude can
76 change during the menstrual cycle (Abitbol, Abitbol, & Abitbol, 1999; Brodnitz, 1979),
77 including differences between high- and low-fertility days (Bryant & Haselton, 2009; but see
78 Fischer et al., 2011). In non-human primates, initial evidence supported a link between sexual
79 calls and female fertility (Tonkean macaques, *Macaca tonkeana*: Aujard et al., 1998; chacma
80 baboons, *Papio ursinus*: O'Connell & Cowlshaw, 1994; Barbary macaques, *Macaca sylvanus*:
81 Semple & McComb, 2000; yellow baboons, *Papio cynocephalus*: Semple, McComb, Alberts, &
82 Altmann, 2002). However, more recent studies, often based on more detailed acoustic analysis of
83 call structure and precise determination of the timing of ovulation through analysis of female
84 reproductive hormones, have found that these calls do not reliably reflect female fertility and
85 ovulation (bonobos, *Pan paniscus*: Clay & Zuberbühler, 2011; long-tailed macaques, *Macaca*
86 *fascicularis*: Engelhardt, Fischer, Neumann, Pfeifer, & Heistermann, 2012; Barbary macaques:
87 Pfefferle et al., 2008; chimpanzees, *Pan troglodytes schweinfurthii*: Townsend, Deschner, &
88 Zuberbühler, 2011). While sexual swellings in these species tend to be graded signals of fertility
89 that provide probabilistic information regarding the likelihood of conception, it seems that in at
90 least some cases sexual calls are less reliable in this regard. That these signals are produced in
91 different modalities suggests that they may function to provide different audiences with different
92 information (Liebal et al., 2013) regarding female mating behavior and fertility. However, in
93 addition to being limited to catarrhines, these studies on sexual calls have largely focused on
94 copulation calls. Little is known about the function of other kinds of sexual calls that are uttered

95 during pre- and post-copulatory contexts (but see Aujard et al., 1998), and little work has been
96 done on sexual signals in other primate taxa.

97 Robust (or tufted) capuchin monkeys (genus *Sapajus*) represent an ideal study system to
98 explore the adaptive significance of sexual calls because females in this genus signal sexual
99 proceptivity via a rich repertoire of visual sexual signals, as well as through distinct vocalizations
100 uttered almost exclusively around the period of female behavioral proceptivity (including estrous
101 calls produced while females closely follow a target male, during post-copulatory displays, and
102 during copulation) (Carosi & Visalberghi, 2002; Di Bitetti & Wheeler, 2017). Interestingly, the
103 production of both visual and vocal components of these proceptive displays in black capuchin
104 females (*Sapajus nigritus*) have been shown to be significantly higher during female
105 periovulatory periods than pre- or post-periovulatory periods (Tiddi, Wheeler, & Heistermann,
106 2015). However, while the rate of production of the visual components of the proceptive displays
107 have been demonstrated to be a probabilistic signal of ovulation to males (i.e. proceptive displays
108 gradually increase in frequency with the approach of ovulation), the rate of production of non-
109 copulatory sexual calls did not raise accordingly with the approach of ovulation (Tiddi et al.,
110 2015). It remains unclear, however, whether their acoustic structure could change in relation to
111 the timing of ovulation, thus potentially providing listeners with information about the timing of
112 the female fertile phase.

113 The aim of the present study was to explore the association between female fertility and
114 the acoustic parameters of female sexual calls in wild black capuchin monkeys (*Sapajus*
115 *nigritus*). Firstly, we describe the repertoire of sexual calls given by females, and test whether
116 there are differences between call bouts given in pre- and post-copulatory contexts. Then, we
117 assess whether changes in female reproductive state, as determined by analysis of female ovarian

118 hormones, are associated with changes in the acoustic parameters of sexual calls by comparing
119 calls given during the periovulatory phase (fertile phase) with calls given during non-
120 periovulatory phase (non-fertile phase). Finally, we tested whether, within the periovulatory
121 phase, the temporal or spectral acoustic parameters of the calls varied based on the timing of
122 ovulation (i.e., days relative to ovulation).

123

124 **Methods**

125 *Study site and subjects*

126 The data for this study were collected in Iguazú National Park, Argentina (25°40' S, 54°
127 30'W). The study area comprises a protected area of 60000 ha in the Upper Parana Atlantic
128 Forest, constituted by semi-deciduous secondary forest with a humid subtropical climate (Di
129 Bitetti, Placci, & Dietz, 2003).

130 Black capuchin monkeys are medium-sized (2.5-3.5kg), diurnal, arboreal and
131 omnivorous primates (Brown & Zunino, 1990; Smith & Jungers, 1997). Black capuchins at the
132 field site live in stable multimale-multifemale groups ranging from 7 to 44 individuals (Janson,
133 Baldovino, & Di Bitetti, 2012). Each group has a clear alpha male that is spatially and socially
134 central in the group and holds feeding and mating priority (Janson, 1990; Tiddi, Aureli, Schino,
135 & Voelkl, 2011; Tiddi et al., 2015). Females remain in their natal groups, whereas males disperse
136 upon reaching sexual maturity (Janson et al., 2012). Females in this population show strong
137 mating seasonality (from May to August) with births occurring from the beginning of October to
138 the end of February (Di Bitetti & Janson, 2001).

139 Robust capuchin females (*Sapajus spp.*) lack any external morphological sexual signals.
140 However, during their proceptive periods (average length: 4-5 days), they display a rich and
141 elaborate repertoire of sexual behaviors that consists of both visual (postures, gestures and facial

142 expressions) and vocal components (Carosi, Heistermann, & Visalberghi, 1999; Carosi &
143 Visalberghi, 2002; Di Bitetti & Wheeler, 2017; Matthews, 2012; Tiddi et al., 2015). Although
144 detailed acoustic analysis has not been conducted on these vocalizations, a recent analysis of
145 most of the calls in the vocal repertoire of black capuchins noted that these sexual calls are easily
146 distinguishable by ear from other call types (Di Bitetti & Wheeler, 2017). When females are
147 proceptive, they actively initiate sexual interactions and persistently solicit a male; while this is
148 most frequently the alpha male, females mate promiscuously and copulations with lower-ranking
149 males are common (Janson, 1984; Lynch Alfaro, 2005). After ejaculation has occurred, males
150 and females can display intense reciprocal post-copulatory courtship in which both individuals
151 produce vocal and visual signals like those produced by proceptive females (Carosi, Linn, &
152 Visalberghi, 2005; Carosi & Visalberghi, 2002).

153

154 *Fecal sample collection, hormone analysis and definition of female fertile phase*

155 During the entire study, we observed 72 periods of female proceptivity, and we were able
156 to match acoustic data with hormonal profiles in 26 periods from 12 females. Fecal samples for
157 monitoring female reproductive state were collected opportunistically (between 7:00 and 18:00)
158 and with sufficient regularity to allow a reliable determination of the timing of ovulation. Fecal
159 sample collection and extraction of hormone metabolites in the field laboratory was performed
160 following the procedure described in Wheeler et al. (2013; 2014). As in Tiddi et al. (2015), the
161 assessment of female fertile phase and the timing of ovulation were based on the pattern of
162 progesterone metabolites excreted in feces (N=409 fecal samples). Fecal progestogen levels were
163 determined using a validated enzyme immunoassay as described in detail in Tiddi et al. (2015).
164 Intra- and inter-assay coefficients of variation of high- and low-value quality controls measured

165 within and between assays were 6.9% and 14.8% (high) and 7.7% and 16.0% (low), respectively.
166 Based on a short excretion time of fecal hormone metabolites more typical of urinary hormone
167 metabolites (Carosi et al., 1999; Wheeler et al., 2013), we defined a 2-day window in which
168 ovulation was most likely to occur, consisting of the day of the defined rise in fecal progesterone
169 levels and the day before (Tiddi et al., 2015). The fertile phase (peri-ovulatory period, POP) of
170 each ovarian cycle was then defined as the period comprising the 2 days of the ovulation window
171 plus two preceding days (from day -2 to day 0 from ovulation), in order to account for sperm life
172 span in the female reproductive tract (Brauch et al., 2007). In addition, the 3-day periods
173 preceding and following the fertile phase were defined as the pre-fertile phase (pre-POP) and
174 post-fertile phase (post-POP), respectively.

175

176 *Sexual calls, collection of recordings and acoustic analysis*

177 For the purpose of this study, we divided sexual calls into three contextual types
178 according to the temporal occurrence of the specific call in relation to mating. “Estrous calls”
179 were uttered before copulation while females solicit and follow a target male, “copulation calls”
180 were given during mating events, and “post-copulatory calls” were emitted during post-
181 copulatory courtship when both the female and the male reciprocally produce similar vocal and
182 visual signals while oriented toward one another (Carosi et al., 2005; Janson, 1984). Females
183 often reinitiated following and soliciting the male after the post-copulatory display, at which
184 point calls were again considered “estrous calls”.

185 Acoustic recordings were collected opportunistically over 5 consecutive mating seasons
186 (2010 to 2014) from early May until late August, on 12 sexually mature, individually-recognized
187 females belonging to three well-habituated groups (Macuco, Rita and Spot groups). Female
188 sexual calls were audio recorded at a 16-bit resolution and a 44.1 kHz sampling frequency using

189 a Sennheiser ME-67 or ME-66 directional microphone coupled with a K6 amplifier and
190 connected to a Marantz PMD-660 or PMD-661 audio recorder, generally at a distance of 3-10 m
191 from the caller.

192 We collected over 500 recordings, but only a subgroup of these could be matched with
193 hormonal data (26 ovarian cycles in total) and therefore used for the main analysis. Accordingly,
194 we included 154 recordings of estrous calls from 12 study females and 56 recordings of post-
195 copulatory calls from 9 females.

196 Sexual call recordings were imported into Avisoft SASLab Pro 5 (R. Specht, Berlin,
197 Germany), where the sampling frequency was converted to 22.05 kHz at 16-bit resolution. We
198 then generated frequency-time spectrograms using a fast Fourier transformation (512-pt FFT,
199 Hamming window with 87.5% overlap and time resolution of 2.9ms). To carry out the acoustic
200 analysis, we started by sampling single 30-sec fragments from each recording available, and only
201 when a recording was longer than 5 minutes we additionally selected 30 seconds at the end of it.
202 Spectrograms from these 30-sec fragments were visually inspected, and any fragments that
203 showed interference of calls from conspecifics or heterospecifics was not further considered.
204 These fragments (including both estrous calls and post-copulatory calls) were composed of
205 multiple bouts, which in turn consisted of several call units (mean \pm SE = 52.7 ± 11.7 call units
206 per fragment), elsewhere termed “notes” (Geissmann, 2002) or “syllables” (Robinson, 1984;
207 Wheeler & Hammerschmidt, 2013). Different call units were visually inspected and categorized
208 based on the frequency contours of the first harmonic on the spectrogram (see Results for further
209 details). Each call unit was generally clearly distinct from others based on visual inspection of
210 spectrograms. We defined a bout as a vocal utterance consisting of either a single unit or a
211 temporal sequence of several units. The delay between two consecutive call units within a bout

212 was by definition shorter than the delay between two consecutive bouts (see Figure S1 in the
213 ESM). In those cases in which visual identification of call units was complicated by the scatter
214 structure of the recording (i.e., the interval between two consecutive calls consisted of several
215 seconds), we applied a 0.20 sec interval between two consecutive units in order to define a call
216 bout. This interval was chosen as appropriate temporal threshold when visually inspecting our
217 spectrograms (the typical interval separating units within clearly-identifiable bouts was < 0.10
218 sec) and at the same time measuring the delay between some obvious bouts. For every 30-sec
219 fragment, we measured a total of 22 structural/temporal call parameters (e.g., unit type, unit
220 delivery rate; unit interval, unit duration) in our three types of calls using Avisoft SASLab Pro 5
221 (see Table 1 for a complete description of these parameters).

222

223 *Data analysis*

224 To characterize the repertoire of sexual calls and determine if estrous calls could be
225 distinguished from post-copulatory calls, we performed a 2-step cluster analysis (using IBM
226 SPSS 24) based on call type, call duration, number of calls per bout, and number of different
227 calls per bout. We included 1373 calls of 627 call bouts from nine subjects in the cluster
228 analysis. To test if the use of bouts belonging to the three resultant clusters (see Results) differs
229 across our *a priori* context-based call categories (i.e., estrous calls versus post-copulatory calls),
230 we first calculated the percent of call bouts from each context that fall into each cluster for each
231 of the nine subjects, and then conducted three Wilcoxon signed ranks tests (one for each cluster)
232 comparing the proportion of estrous and post-copulatory calls that fall into each cluster (using
233 IBM SPSS 25).

234 To test if the variables we measured on call bouts co-varied with female fertility, we first
235 reduced the number and correlation between the different acoustic measurements by performing

236 a principal component analysis (PCA) for estrous calls as well as for post-copulatory calls. We
237 included in the subsequent analysis all factors with an eigenvalue above 1. To get a better
238 interpretation of factor loadings, we did a subsequent Varimax rotation. We then tested for a
239 relation between these principal component values and female fertile phase using within-subject
240 linear regressions. These fixed effect models were based on a within-subject centering approach
241 which allow one to assess whether the between-subject effect differs from the between-subject
242 effect of the covariate, while accounting for unequal sample sizes and repeated observations of
243 the same subjects (van de Pol & Wright, 2009). Using these models, we analyzed the effect of
244 female fertile phase on acoustic parameters characterizing female estrous and post-copulatory
245 calls. To do so, we ran different within-subject linear regressions where components extracted
246 from the PCA were entered as the response variable and female fertile phase as the main
247 explanatory variable, with the latter considered in two different ways. First, we ran an initial set
248 of models in which the response variable was the selected factors from the PCA for estrous calls
249 (7 in total, see Table S1 in the ESM) and the independent variable was a categorical variable
250 representing days within the periovulatory period (POP, fertile phase) or within the non-
251 periovulatory period (non-POP, non-fertile phase). Non-periovulatory periods included both the
252 3-day period preceding the fertile phase (pre-POP) and the 3-day period following it (post-POP)
253 in which females showed sexual displays. Because only a small number of post-copulatory calls
254 were recorded during non-POP periods, it was not possible to compare the acoustic structure of
255 these calls between fertile and non-fertile periods. Female identity was entered as a fixed effect
256 in all models.

257 As second step, in order to test whether any parameters of the calls gradually changed
258 within the periovulatory period (POP) with the approach of the ovulation, we ran addition

259 within-subject linear regressions in which the response variables were the selected factors
260 extracted by the PCA, and the independent continuous variable was the number of days to
261 ovulation within the POP. In this case, given the sufficient number of recordings of both estrous
262 calls and post-copulatory calls within the fertile period, we ran these models separately for the
263 two call types (estrous calls: 7 extracted factors; post-copulatory calls: 6 extracted factors; see
264 Tables S1 and S2 in the ESM, respectively). As above, female identity was entered as a fixed
265 effect.

266 Principal component analysis was performed using IBM SPSS 21 and within-subject
267 linear regressions were run in R version 3.2.1. All analyses were two-tailed and the significance
268 level was set at $P < 0.05$. We applied a Bonferroni correction for multiple testing, therefore setting
269 the significance at 0.007 on the models for the effect of cycle stage (POP/non-POP) on estrous
270 calls, as well as on the models for the effect of the day to ovulation on estrous calls, and at 0.008
271 on the models for the effect of day to ovulation on post-copulatory calls.

272

273 **Ethics Guidelines**

274 This study was approved by the Animal Welfare Officer at the German Primate Center
275 (DPZ) and by the Argentine Administration of National Parks (permit number: NEA 158 bis Rnv
276 5), and adhered to the legal requirements of Argentina. Finally, this research adhered to the
277 American Society of Primatologists' Principles for the Ethical Treatment of Primates.

278

279 **Results**

280 *Description of repertoire of sexual calls*

281 Based on visual inspection of spectrograms, we identified seven acoustically distinct call
282 units that were produced during bouts of estrus calling (labelled “A” calls, “B” calls, and so on
283 through “G” calls; see Figure 1 for an example of visual identification of the different call units).
284 Some of these seven call types show some acoustic similarities to other call types in the black
285 capuchin vocal repertoire previously described for non-sexual contexts (Di Bitetti & Wheeler,
286 2017). “A” calls show structural similarities to some variants of calls termed “whistle series”,
287 particularly the “infant whistle series” and the “intergroup encounter whistle series,” but has
288 shorter and more widely spaced call units than does the either, is of lower frequency than the
289 former, and is of far lower amplitude than the latter. “B” calls are most similar to what Di Bitetti
290 and Wheeler (2017) referred to as “piripipi estrous calls”, which they noted share some
291 similarities to calls (“sirenas”) given by same-group males reuniting after periods of separation
292 (Lynch Alfaro, 2008). The concave and upward sweeping frequency contours of “C” and “D”
293 calls, respectively, are unique compared to other calls from the repertoire that have been
294 described. Individual units of the “E” call, characterized by a convex frequency contour, are
295 similar to what has been called the “contact note”, although “E” calls appear to be higher in
296 frequency and are given in bouts with rapid repetition of the unit, whereas repeated contact notes
297 are much more widely spaced; the rapid repetition of these units within bouts is similar the
298 “aggressive contact note” given in agonistic contexts, although “E” calls are again higher in
299 frequency. The waveform contour of “F” calls are similar to “squeals”, and this call may grade
300 into the “sirena” reunion calls mentioned above. Finally, “G” calls are the noisiest calls in the
301 repertoire of sexual calls, and are most similar to “screams”, but appear to be lower amplitude
302 and are usually characterized by shorter unit durations than is typical of screams, although unit

303 duration varies and may be longer immediately before and during copulation, although further
304 study is needed to confirm this (see also Di Bitetti & Wheeler, 2017)

305 Running a 2-step cluster analysis based on call type, call duration, number of calls per
306 bout, and number of different calls per bout, we found support for a three cluster solution,
307 indicating that calls could be considered to fall into one of three categories. Calls belonging to
308 the first cluster (30.3%) were mostly single calls or bouts of the same call type and comprised the
309 call types “A”, “B”, “D”, “E” and “F”. The second cluster (20%) consisted nearly exclusively of
310 “F” and/or “G” call types (only a small proportion of “G” calls did not fall in this cluster”). The
311 third cluster (49.7%) consisted of bouts characterized by different call types.

312

313 *Contextual variation in call production*

314 In testing whether estrous and post-copulatory calls differed in terms of cluster usage, we
315 found no difference between these calls in terms of the proportion of each that belonged to
316 cluster 3 (two-tailed Wilcoxon Signed Ranks Test: $T^- = 19$, $N = 9$, $p = 0.734$; Figure 2). In
317 contrast, for all nine subjects, a greater proportion of their estrous calls than their post-copulatory
318 calls fell into cluster 1 ($T^+ = 0$, $N=9$, $p > 0.01$; Figure 2), while for 7 of 9 subjects a greater
319 proportion of their post-copulatory calls than their estrous calls fell into cluster 2 ($T^- = 3$, $N = 9$,
320 $p = 0.02$; Figure 2). Spectrograms of typical estrous and post-copulatory calls, together with a
321 typical copulation call, can be found in Figures 3a-d.

322

323 *Estrous calls in relation to the ovarian cycle phase and the timing of ovulation*

324 Principal components analysis gave seven axes for estrous calls, accounting for 84.2% of
325 the variability in the data. The factor loadings (Table S1 in the ESM) indicated that the different

326 factors mainly described: (1) unit delivery rate, (2) unit duration, (3) noisiness, (4) upward
327 sweeping units, (5) convex frequency modulated units, (6) concave frequency modulated units,
328 and (7) flat frequency units. Results show that whether a female was in her fertile phase (POP)
329 or not (non-POP) did not significantly affect any of the PCA components (Table 2a; Fig. 4a).
330 Similarly, when considering only data collected within the periovulatory period (POP), none of
331 the PCA components varied significantly with the approach of ovulation (Table 2b; Fig. 4b).

332

333 *Post-copulatory calls in relation to the timing of ovulation*

334 Six factors were extracted from the principal components analysis for post-copulatory
335 calls (Table S2), accounting for 85.4% of the variability. Factors mainly represent: (1) unit
336 delivery rate, (2) unit duration, (3) noisiness and flat frequency units, (4) upward sweeping and
337 convex frequency modulated units, (5) concave frequency modulated units and (6) bout delivery
338 rate. When considering the day to ovulation within the fertile phase (POP), our results indicated
339 that none of the PCA components were associated with the approach of ovulation (Table 3; Fig.
340 5).

341

342 **Discussion**

343 The results of the present study provide details on the sexual call repertoire of black
344 capuchin monkey females and show that patterns of estrous and post-copulatory calls form three
345 distinct clusters, with calls falling into one of these clusters being more associated with pre-
346 copulatory contexts, the second being more associated with post-copulatory contexts, and the
347 third being associated with both contexts. However, our results indicate that there is no
348 relationship between female fertile phase and the characteristics of sexual calls. Specifically,
349 although mainly produced around fertile periods (Tiddi et al., 2015), we did not find any

350 significant difference between acoustic parameters of estrous and post-copulatory calls given
351 during the periovulatory (i.e., fertile) period (POP) and those given during the non-periovulatory
352 period (non-POP). Furthermore, when only sexual calls occurring within the periovulatory
353 period were considered, none of the acoustic parameters changed in relation to the timing of
354 ovulation. Thus, the acoustic structure of estrous and post-copulatory calls do not appear to have
355 the potential to provide receivers with information regarding either the caller's fertility state or
356 the timing of ovulation within the fertile period.

357 The results of this study indicating that the use of sexual calls varies probabilistically
358 based on context (i.e., between pre-copulatory estrous calls and post-copulatory displays) but not
359 on female fertility corroborates previous work indicating that these signals contrast with visual
360 signals in the extent to which they provide reliable information on black capuchin females' states
361 of fertility. More broadly, our results also contrast with early evidence indicating that sexual calls
362 function as signals of female fertility in primates (e.g., Aujard et al., 1998), and add to the
363 evidence that covariation of the acoustic structure of sexual calls with female fertility might be
364 less common than initially thought among primates (Brauch et al., 2007; Clay & Zuberbühler,
365 2011; Engelhardt et al., 2012; Townsend et al., 2011). The lack of such covariation has been
366 argued to be related to the fact that primate females are usually characterized by elevated levels
367 of estrogens for only a few days during the ovulatory cycle, and this brief rise in estrogens may
368 be sufficient to induce the production of sexual calls, but not sufficient to cause an up-regulation
369 of hormone receptors in the sound production organs (Pfefferle et al., 2011). This may be the
370 case here, as the occurrence of estrous calls in black capuchin females is much more common
371 during a female's fertile period (Tiddi et al., 2015), but we did not find any fine-tuned
372 differences in the acoustic structure of these calls with the approach of ovulation. In other words,

373 levels of estrogens above a given threshold may stimulate the production of estrous calls, but
374 variation above the threshold may not cause additional changes in the acoustic characteristics of
375 these calls. It should be noted, however, that the production of visual signals of fertility (facial
376 expressions and gestures) by female black capuchins do show variation in relation to the timing
377 of ovulation and thus variation above this threshold of estrogen levels (Tiddi et al., 2015). Future
378 hormonal analysis focused on detailed changes in estrogens during the female ovarian cycle may
379 provide insight into these different responses of signal modalities to the approach of ovulation.

380 Although our study could not assess the function of copulation calls, the absence of
381 precise cues of fertility in black capuchin female estrous and post-copulatory calls indicate that
382 these vocalizations may serve to advertise mating to other group males, and thus motivate the
383 target male to invest in mate-guarding. Within this context, capuchin females may benefit by
384 gaining repeated mating with the target male and, therefore, increase the probability of
385 fertilization by males with good genes (Maestripieri & Roney, 2004). Alternatively, given the
386 prevalence of sexually selected infanticide in our study population (Ramirez-Llorens, Di Bitetti,
387 Baldovino, & Janson, 2008), advertising mating may provide females with the advantage of
388 increasing the confidence of paternity in alpha males, who in turn may be more motivated to
389 engage in infanticide deterrence behaviors (Henzi, 1996). The lack of fertility cues in sexual calls
390 may also be explained by considering a more active role of females in choosing their mates
391 (O'Connell & Cowlshaw, 1994). In this scenario, where females and males face a conflict of
392 reproductive interest, female vocalizations may incite male sperm competition by eliciting the
393 interest of other group males, and mating males may respond by mate-guarding females. Indeed,
394 previous work on robust capuchins has suggested that male post-copulation courtship may
395 represent a form of mate guarding (Carosi et al., 2005; Lynch Alfaro, 2005). It is important to

396 note, however, that overall these different functions of copulation calls are not mutually
397 exclusive and that females may fully benefit by combining behaving in a way that both increases
398 confidence of paternity in dominant males and at the same time confuses paternity across
399 multiple males (Zinner, Nunn, van Schaik, & Kappeler, 2004). The current findings, combined
400 with previous results on visual signals and call production patterns, suggest that black capuchin
401 females may in fact apply such combined behavior; on the one hand, they increase paternity
402 confidence in the alpha male via visually conveying to him graded signals of fertility, while on
403 the other hand confusing paternity by encouraging promiscuous mating by varying the structure
404 of their calls only according to pre- and post-copulatory contexts, but not indiscriminately
405 broadcasting fertility via vocal signals. This scenario seems plausible in our study population,
406 wherein females show a high preference for the alpha male in their group, but still mate
407 promiscuously with other group males, and face a relatively high risk of infanticide during
408 within-group male take-overs (Janson et al., 2012).

409 Further support for the idea that females may be balancing paternity confusion across
410 multiple males while concentrating paternity certainty in the alpha male is provided by the
411 evidence that capuchin females may use multimodal sexual signals to advertise their fertility
412 with different degrees of accuracy (Higham et al., 2012; Rigaille, Higham, Lee, Blin, & Garcia,
413 2013; Tiddi et al., 2015). As sexual calls are almost exclusively produced in association to the
414 fertile period (Carosi et al., 1999; Tiddi et al., 2015), females may use them to advertise their
415 general fertility state to all group males, without conveying precise information regarding the
416 timing of ovulation. Such precise information is provided, instead, by facial expressions and
417 gestures (Tiddi et al., 2015), which are typically visible only to neighboring individuals due to
418 vegetation density (see Liebal et al., 2013). Such non-redundant multi-modal signaling (see

419 Partan & Marler, 1999) via visual and vocal components may enable females to provide only a
420 general and less accurate signal of their fertility to subordinate peripheral males via sexual calls,
421 while more precise signals related to the timing of ovulation is conveyed mainly via visual
422 proceptive displays which are only received by the target males in close proximity. Such non-
423 redundant multimodal signals may facilitate mating with multiple males when conception is less
424 likely, while also concentrating paternity certainty in the dominant male by encouraging
425 monopolization by them during the days in which mating is most likely to lead to conception
426 (Tiddi et al., 2015).

427 Future work is needed to better understand the acoustic variation that exists within and
428 between estrous calls, copulation calls, and post-copulatory calls, and the causes of this variation.
429 Although systematic acoustic analysis is lacking, previous work has described black capuchin
430 copulation calls as being acoustically similar to the noisy estrous calls given in conjunction with
431 higher intensity visual displays, but distinct from those given nearly continuously by estrous
432 females when following males (Di Bitetti & Wheeler, 2017). Anecdotal observations in the
433 current study suggest that such noisy pre-copulatory calls are relatively rare, and it remains
434 unclear the extent to which listeners can reliably determine the occurrence of a copulation based
435 solely on these calls. Given the lack of an association between female fertile state and acoustic
436 parameters in the current study, together with the anecdotal observation that call structure seems
437 to vary with both the occurrence of copulations and the intensity of the visual displays, it seems
438 likely that acoustic differences in these calls may be proximately related to female arousal.
439 Additional research is needed to test this hypothesis, and the factors other than copulation that
440 contribute to variation in female arousal.

441 In conclusion, our results indicate that black capuchin females do not provide precise
442 information about their fertile period or the timing of ovulation via changes in the acoustic
443 structures of their sexual calls. However, as these calls are almost exclusively produced during or
444 immediately before or after a fertile period (Tiddi et al., 2015), females may produce them to
445 advertise their general fertility to group males, without more providing precise information about
446 the likelihood of conception. This, combined with previous findings, suggests that black
447 capuchin females may use different modalities, such as vocal and visual displays to convey
448 information about their fertility with different degrees of accuracy. Similar studies of multimodal
449 sexual signaling across taxa may allow a better understanding of the ecological and social
450 pressures under that shape mating strategies in primates.

451

452 **Conflict of Interest**

453 The authors declare no conflicts of interest.

454

455 **Acknowledgments**

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615

616 **Table 1.** Descriptions of the 22 parameters used in the analysis of estrous and post-copulatory calls.

Parameters	Description
Unit delivery rate	Number of calls units uttered per second
Unit interval	Mean interval between one call unit and the next one [s]
Unit duration	Mean duration from the beginning to the end of the call unit [s]
Flat frequency units delivery rate	Mean number of flat frequency units uttered per second
Proportion of flat frequency units	Mean proportion of flat frequency units in comparison to the total number of call units given.
Downward sweeping units delivery rate	Mean number of downward sweeping units uttered per second
Proportion of downward sweeping units	Mean proportion of downward sweeping frequency units in comparison to the total number of call units given.
Concave units delivery rate	Mean number of concave units uttered per second
Proportion of concave units	Mean proportion of concave units in comparison to the total number of call units given.
Upward sweeping units delivery rate	Mean number of upward sweeping units uttered per second
Proportion of upward sweeping units	Mean proportion of upward sweeping units in comparison to the total number of call units given.
Convex units delivery rate	Mean number of convex units uttered per second
Proportion of convex units	Mean proportion of convex units in comparison to the total number of call units given.
Waveform units delivery rate	Mean number of waveform units uttered per second
Proportion of waveform units	Mean proportion of waveform units in comparison to the total number of call units given.
Noisy units delivery rate	Mean number of noisy units uttered per second
Proportion of noisy units	Mean proportion of noisy units in comparison to the total number of call units given.
Bout rate	Number of bouts uttered per second
Single units/sequences	Ratio of the number of single call units to the number of sequences
Sequence length	Mean duration from the beginning to the end of the sequence [s]

Units per sequence

Mean number of call units in a sequence

Different units per sequence

Mean number of different call units in a sequence

617

618 **Table 2.** Results of the fixed effect models testing the association between sexual hormones and acoustic
619 parameters of estrous calls in black capuchin females. The factors extracted by the Principal Components
620 Analysis (PCA) are used as the dependent variable in the models and either a) cycle stage (perioovulatory
621 period or non-perioovulatory period) or b) day to ovulation as the independent variable. Female identity
622 was entered as a fixed factor.

	Estimate	SE	t value	Pr(> t)
a)				
FAC1 “unit delivery rate”	0.095	0.086	1.109	0.270
FAC2 “unit duration”	0.144	0.101	1.430	0.156
FAC3 “noisiness”	0.129	0.108	1.185	0.239
FAC4 “upward sweeping units”	-0.023	0.100	-0.231	0.818
FAC5 “convex units”	0.164	0.110	1.492	0.139
FAC6 “concave units”	-0.127	0.106	-1.205	0.231
FAC7 “flat frequency units”	-0.118	0.107	-1.109	0.270

N = 154 recordings from 12 females. Female identity was entered as a fixed factor.

b)				
FAC1 “unit delivery rate”	0.512	0.260	1.973	0.051
FAC2 “unit duration”	-0.335	0.291	-1.152	0.251
FAC3 “noisiness”	-0.387	0.297	-1.301	0.196
FAC4 “upward sweeping units”	-0.113	0.270	-0.417	0.677
FAC5 “convex units”	0.301	0.294	1.027	0.306
FAC6 “concave units”	-0.393	0.318	-1.236	0.219
FAC7 “flat frequency units”	0.394	0.295	1.334	0.184

N = 124 recordings from 12 females. Female identity was entered as a fixed factor.

623 **Table 3.** Results of the fixed effect models assessing the effect of sexual hormones on acoustic
 624 parameters of post-copulatory calls in black capuchin females. The factors extracted by the Principal
 625 Components Analysis (PCA) are used as the dependent variable in the models and day to ovulation as the
 626 independent variable.

	Estimate	SE	t value	Pr(> t)
FAC1 “unit delivery rate”	-0.183	0.120	-1.521	0.135
FAC2 “unit duration”	0.231	0.115	2.007	0.051
FAC3 “noisiness and flat frequency units”	-0.028	0.135	-0.206	0.838
FAC4 “upward sweeping and convex units”	-0.281	0.132	-2.123	0.039
FAC5 “concave units”	0.015	0.154	0.100	0.921
FAC6 “bout delivery rate”	0.141	0.145	0.970	0.337

N = 56 recordings from 9 females. Female identity was entered as a fixed factor.

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628

629 **Figure Legends**

630 **Figure 1.** Spectrograms showing visual identification and categorization of the 7 acoustically-
631 distinct units that characterize sexual calls (“A” calls through “G” calls) based on their frequency
632 contours. See Methods for spectrogram parameters.

633

634 **Figure 2.** Bar chart showing mean (\pm SE) proportion of estrous and post-copulatory calls that fell
635 into each of the three clusters for nine females.

636

637 **Figure 3.** Time-frequency spectrograms illustrating typical female sexual calls. (a) Estrous calls,
638 (b) Copulation calls, and two Post-copulatory calls given (c) immediately after copulation and
639 (d) around the middle/end part of the courtship (bottom), when they become similar estrous calls.

640 See Methods for spectrogram parameters.

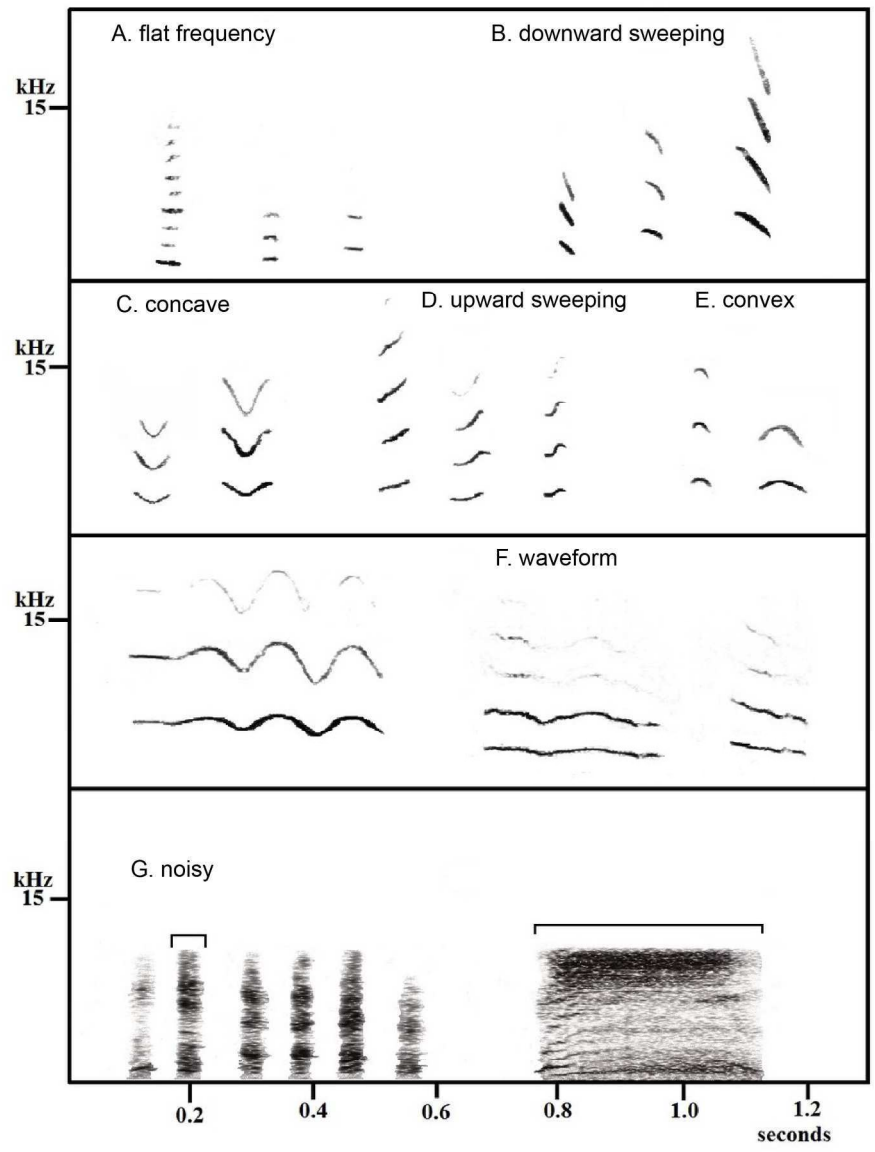
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642 **Figure 4.** Boxplots showing Factor 1 (“unit delivery rate”) for estrous calls: a) during non-fertile
643 phase (non-POP) and fertile phase (POP), and b) with respect to the day relative to ovulation
644 within the fertile phase. Boxplots show median (dark line), 1st and 3rd quartiles (box) and range
645 (whiskers).

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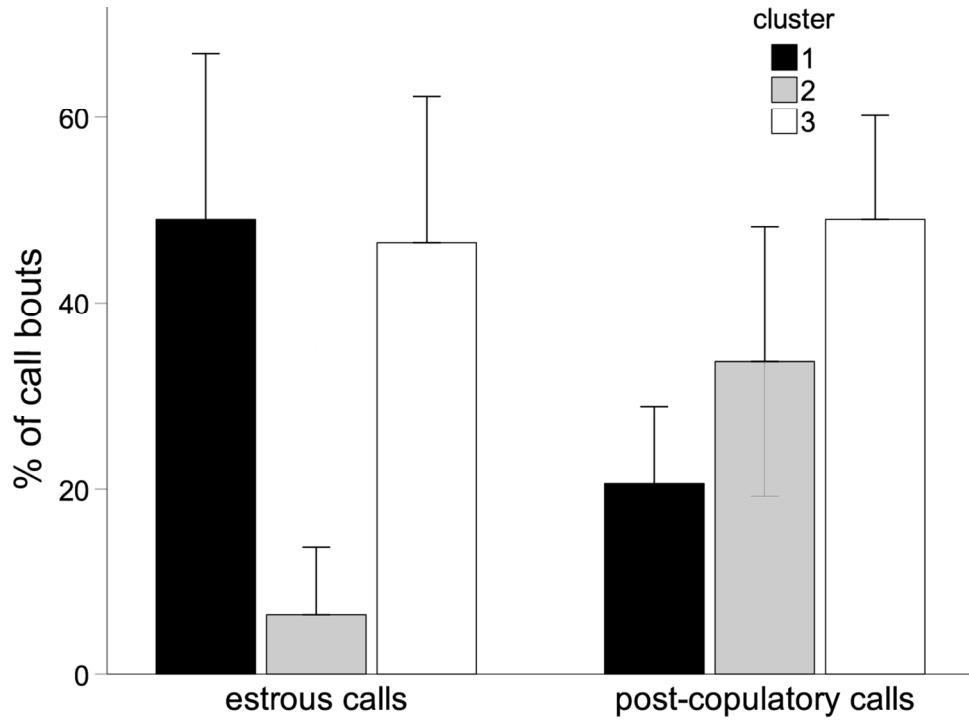
647 **Figure 5.** Boxplots showing Factor 1 (“unit delivery rate”) for post-copulatory calls in relation to
648 the day relative to ovulation within the fertile phase. Boxplots show median (dark line), 1st and
649 3rd quartiles (box) and range (whiskers).

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338x446mm (150 x 150 DPI)

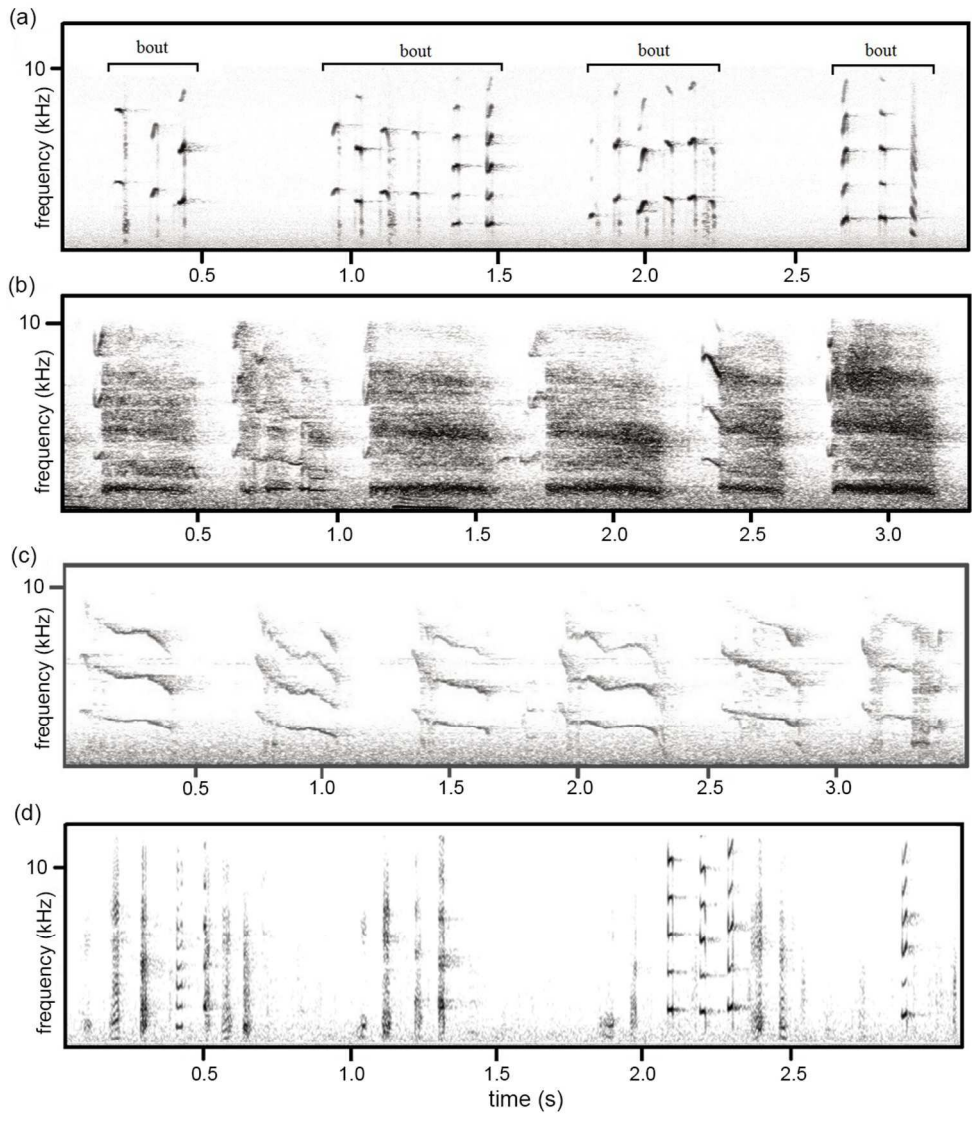
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227x162mm (150 x 150 DPI)

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330x378mm (96 x 96 DPI)

Fig.3
a)

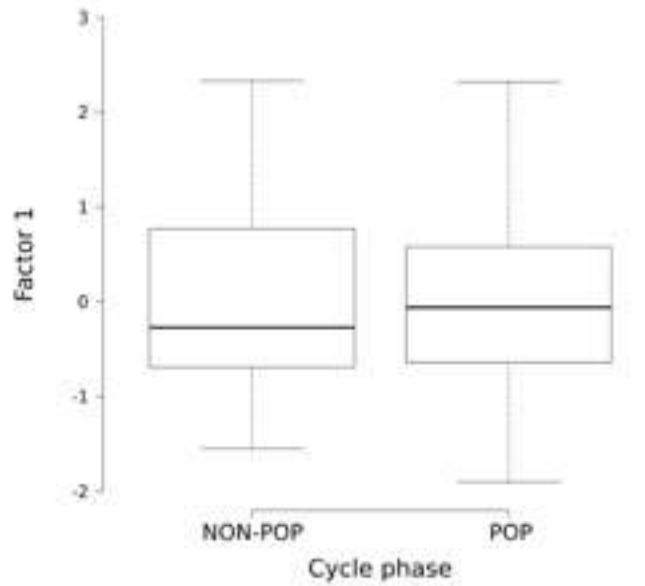


Fig.3
b)

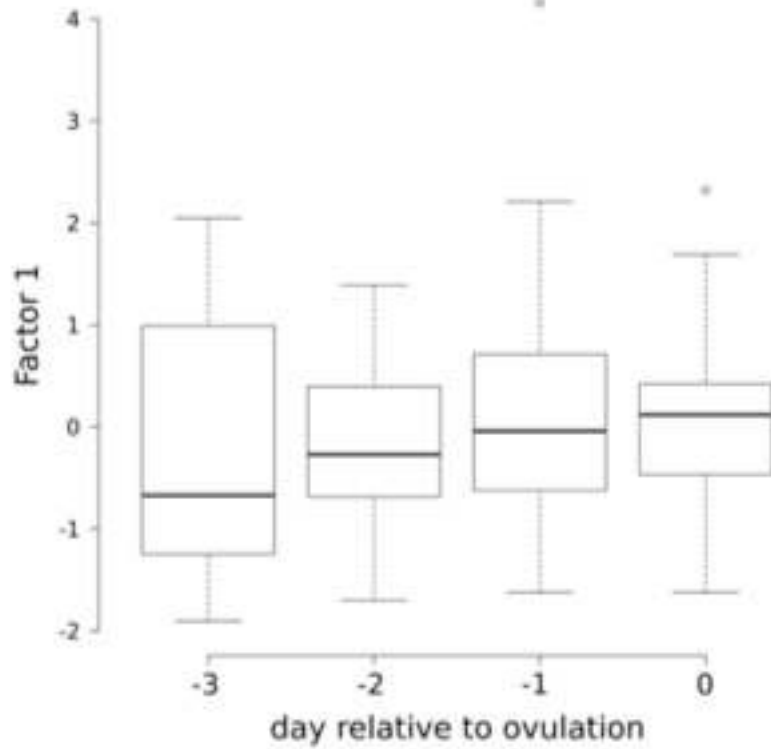
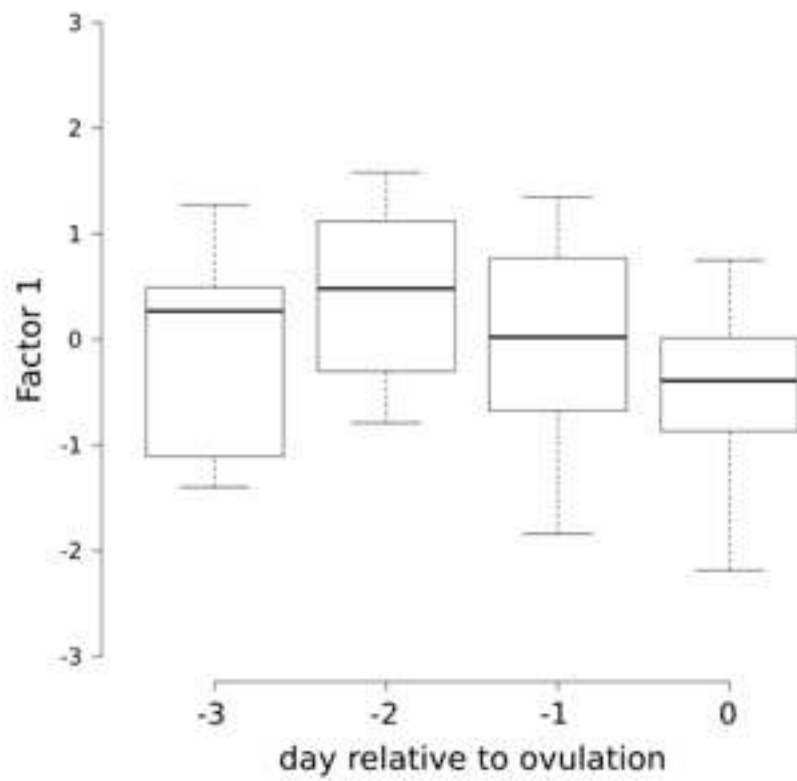


Fig. 4



1 Supplementary Materials for

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3 **Do sexual calls in female black capuchin monkeys (*Sapajus nigritus*) vary with**
4 **fertility? An acoustic analysis**

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7 Ester Bernaldo de Quirós, Brandon C. Wheeler, Kurt Hammerschmidt, Michael
8 Heistermann, Barbara Tiddi

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11 **Table S1.** Factor loadings from the Principal Component Analysis (PCA) for estrous
12 calls after Varimax rotation. Only factor loadings higher than 0.4 are shown.
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Acoustic parameters	Factors						
	1	2	3	4	5	6	7
Unit delivery rate	0.701						
Unit interval	-0.759						
Unit duration		0.931					
Flat frequency units delivery rate	0.701						0.558
Proportion of flat frequency units							0.948
Downward sweeping units delivery rate	0.902						
Proportion of downward sweeping units			-0.483	-0.533			-0.479
Concave units delivery rate						0.915	
Proportion of concave units						0.946	
Upward sweeping units delivery rate				0.668			
Proportion of upward sweeping units				0.902			
Convex units delivery rate	0.479				0.776		
Proportion of convex units					0.889		
Waveform units delivery rate		0.867					
Proportion of waveform units		0.937					
Noisy units delivery rate			0.915				
Proportion of noisy units			0.961				
Bout rate	0.714						
Single units/sequences	-0.579						
Sequence length	0.733						
Units per sequence	0.797						
Different units per sequence	0.485			0.541			
% of total variance	26.357	12.934	10.953	10.010	8.579	8.335	7.031
% cumulative variance	26.357	39.291	50.245	60.255	68.834	77.169	84.200

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15 **Table S2.** Factor loadings from the Principal Component Analysis (PCA) for post-copulatory
16 calls after Varimax rotation. Only factor loadings higher than 0.4 are shown.

Acoustic parameters	Factors					
	1	2	3	4	5	6
Unit delivery rate	0.866					
Unit interval	-0.765					
Unit duration		0.882				
Flat frequency units delivery rate	0.445		0.648			
Proportion of flat frequency units			0.679			
Downward sweeping units delivery rate	0.707	-0.545				
Proportion of downward sweeping units		-0.685	-0.651			
Concave units delivery rate					0.917	
Proportion of concave units					0.915	
Upward sweeping units delivery rate				0.758		
Proportion of upward sweeping units				0.825		
Convex units delivery rate				0.804		
Proportion of convex units				0.859		
Waveform units delivery rate		0.916				
Proportion of waveform units		0.911				
Noisy units delivery rate			0.784			
Proportion of noisy units			0.782			
Bout rate						0.901
Single units/sequences	-0.592					
Sequence length	0.602	0.513				-0.427
Units per sequence	0.857					
Different units per sequence	0.491		0.491	0.441		
% of total variance	19.240	18.735	15.239	14.890	10.561	6.729
% cumulative variance	19.240	37.975	53.214	68.104	78.665	85.394

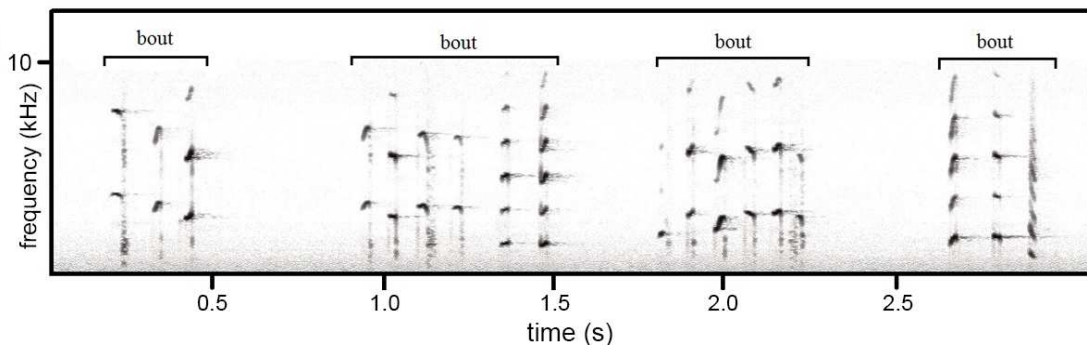
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Figure S1. An example estrous call showing how bouts were defined by inter-unit intervals greater than those between units within bouts.