

Primates

Sexual dimorphism in the loud calls of Azara's owl monkeys (*Aotus azarae*): evidence of sexual selection? --Manuscript Draft--

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Full Title:	Sexual dimorphism in the loud calls of Azara's owl monkeys (<i>Aotus azarae</i>): evidence of sexual selection?	
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Keywords:	vocal communication; loud calls; dimorphism; sexual selection; monogamy; pair-living	
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Funding Information:	Leakey Foundation	MSc Alba Garcia de la Chica Dr. Eduardo Fernandez Duque
	Wenner-Gren Foundation	Dr. Eduardo Fernandez Duque
	National Geographic Society	Dr. Eduardo Fernandez Duque
Abstract:	<p>Primates use different types of vocalizations in a variety of contexts. Some of the most studied types have been the long distance or loud calls. These vocalizations have been associated with mate defense, mate attraction and resource defense, and it is plausible that sexual selection has played an important role in their evolution. Focusing on identified individuals of known sex and age, we evaluated the sexual dimorphism in a type of loud calls (hoots) in a population of wild owl monkeys (<i>Aotus azarae</i>) in Argentina. We found evidence of sexual dimorphism in call structure, with females and males only emitting one type of call, each differing in dominant frequency and Shannon entropy. In addition, both age-related and sex-specific differences in call usage were also apparent in response to the removal of one group member. Future acoustic data will allow us to assess if there are individual characteristics and if the structure of hoot calls presents differences in relation to the social condition of owl monkeys or specific sex responses to variants of hoot calls' traits. This will provide deeper insights into the evolution of vocal mechanisms regulating pair bonding and mate choice strategies in this and other primate species.</p>	
Suggested Reviewers:	Eckhard Heymann EHeymann@dpz.eu	

Response to Reviewers:

Dear Dr. Tetsuro Matsuzawa,

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6 ¹ University of Barcelona, Barcelona, Spain

7 ² The Owl Monkey Project, Formosa, Argentina

8 ³ Universidad Cuenca del Plata, Formosa, Argentina

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11 ⁶ Departamento Zoología Vertebrados, Museo de Historia Natural Noel Kempff Mercado, Santa Cruz
12 de la Sierra, Bolivia

13 ⁷ Department of Anthropology and School of Forestry and Environmental Studies, Yale University,
14 New Haven, CT, U.S.A

15 ⁸ Facultad de Recursos Naturales, Universidad Nacional de Formosa, Argentina

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41

42 **ABSTRACT:**

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44 have been the long distance or loud calls. These vocalizations have been associated with mate defense,
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46 role in their evolution. Focusing on identified individuals of known sex and age, we evaluated the
47 sexual dimorphism in a type of loud calls (hoots) in a population of wild owl monkeys (*Aotus azarae*)
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49 emitting one type of call, each differing in dominant frequency and Shannon entropy. In addition, both
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51 one group member. Future acoustic data will allow us to assess if there are individual characteristics
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55 primate species.

56

57 Key words: vocal communication, loud calls, dimorphism, sexual selection, monogamy, pair-living

58 **Introduction**

59 Vocal communication has been a central topic of interest for primatologists and evolutionary
60 anthropologists for decades (Seyfarth et al. 1980; Snowdon 1989; Ghazanfar and Hauser 1996;
61 Zuberbühler et al. 1999). The study of this type of communication can lead to insights about the
62 evolution and maintenance of the social systems in which they occur (McComb and Semple 2005;
63 Heymann, 2003). Among non-human primates, some of the most studied types of vocalizations have
64 been the long distance or loud calls. Loud calls, like chemical signals, are expected to be relatively
65 more important in arboreal species where visibility may limit the value of visual communication
66 (Epple 1974). In primates, these vocalizations have been associated with resource defense (Sekulic
67 1982; Whitehead 1987; Mitani 1990; Cowlishaw 1996; Steenbeek et al. 1999; Wich and Nunn 2002;
68 Rasoloharijaona et al. 2006), mate attraction (Steenbeek et al. 1999; Wich and Nunn, 2002; Caselli et
69 al. 2018) and infanticide (Wich et al. 2002; Wich et al. 2004). Further, given their important role in
70 maintaining the spatial cohesion of the group, male–female interactions and assisting individuals in the
71 search for potential mates, it is plausible that sexual selection has played an important role in their
72 evolution (Delgado 2006).

73 The formation of a mating pair requires the identification of potential mates, and the ability to
74 differentiate mates from other opposite-sex individuals. Some studies documented variation between
75 the sexes and individual recognition in loud calls in several primate taxa (Benz et al. 1990; Porter
76 1994; Smith et al. 2009; Rukstalis and French 2005; Terleph et al. 2015). In pair-living and sexually
77 monogamous titi monkeys (*Callicebus* spp.) loud calling is commonly emitted as coordinated duets by
78 pairs. Even when no sex-specific duet contributions have been detected (*C. ornatus*, Robinson 1979;
79 *C. cupreus*, Müller and Anzenberger 2002; *C. nigrifrons*, Caselli et al. 2015), it is possible that sex
80 contributions differ in the acoustic characteristics of syllables that are assembled to compose the song

81 parts of duets' long sequences, allowing individuals to identify the sex of the caller (Caselli et al.
82 2015).

83 Another pair-living and sexually monogamous taxon which can serve as a model to examine
84 the possible sex differences of loud vocalizations is *Aotus azarae*, the Azara's owl monkeys of
85 Formosa Province in Argentina. Owl monkeys live in groups composed of a heterosexual pair that
86 mates monogamously (Huck et al. 2014a), one infant, one or two juveniles and sometimes a subadult
87 (Fernandez-Duque 2016). In the owl monkey population we study in Guaycolec Ranch, all male and
88 female subadults disperse from their natal groups and become floaters, who range solitarily while
89 looking for a reproductive position in another group (Huck and Fernandez-Duque 2017). Preliminary
90 analyses indicate that, given the relatively low infant and juvenile mortality, there are more floaters
91 than available breeding positions (Huck and Fernandez-Duque 2017), which leads to an intense and
92 frequent competition over breeding positions for both males and females (Fernandez-Duque and Huck
93 2013). Owl monkeys show extremely low levels of dimorphism in body size, coloration, and the
94 external genitalia, and like other pair-living sexually monogamous taxa, they show an extended form
95 of male care (Huck et al. 2011; Huck and Fernandez-Duque 2012; Spence-Aizenberg et al. 2017;
96 Spence-Aizenberg et al. 2018).

97 Based on Trivers' (1972) hypothesis that the sex with less investment in infant care would be
98 the more competitive one, Heymann (2003) proposed that, among New World Monkeys, the degree of
99 male care is related to the influence of sexual selection on the evolution of chemical and vocal
100 communication. He predicted that in taxa where males were the principal providers of infant care,
101 females would vocalize more than males. In contrast to other genera, our knowledge of owl monkey
102 vocal communication that would allow evaluation of the hypothesis remains quite limited. Only two
103 studies on captive animals have assessed the vocal repertoire of *Aotus* (Moynihan 1964; Kantha et al.

104 2009); in two other studies of vocal communication in wild owl monkeys the authors were not able to
105 unequivocally identify the sexes or age of the individuals due to the strictly nocturnal habits of the
106 species studied (Wright 1985; Helenbrook et al. 2018). These studies suggested that one call, the hoot
107 call, conveys information over long distances and that could be differentiated into two sub-types, graff
108 and tonal ones (Wright 1985).

109 The goal of our study was to assess sex differences in hoot calls in wild owl monkeys. Using
110 only information from identified individuals of known age and sex we first assessed whether hoots are
111 sexually dimorphic, comparing information on the rate of production of graff and tonal hoots. We
112 predicted that tonal and graff hoots would be sex-specific. Second, we compared audio recordings of
113 tonal and graff hoots to test the prediction that there would be acoustic differences in the basic
114 structure of both types of calls. Finally, to examine whether the levels of dimorphism in hoot calls are
115 consistent with the hypothesis proposed by Heymann (2003) we tested the prediction that females
116 would emit more hoot calls than males given the high degree of paternal care in owl monkeys.

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125 **Methods**

126 *Study area and population*

127 The study area, located in the gallery forests along the Pilagá River in the Argentinean Gran
128 Chaco, is part of the 1,500 ha Owl Monkey Reserve established in 2006 (58°13' W, 26° 54'S). The
129 area includes a mosaic of grasslands, savannas, xeric thorn forests and semideciduous forests (van der
130 Heide et al. 2012). The climate is subtropical with no marked wet season (1418mm/yr), and extreme
131 low and high temperatures are frequent (daily minimum temperatures <10°C between April and
132 September and maximum daily temperatures >33°C between September and March; Huck et al. 2017).
133 A system of intersecting transects at 100m intervals covers approximately 300ha of forest where all of
134 the data were collected (Fernandez-Duque 2016).

135 Unlike the strictly nocturnal tropical species of owl monkeys, *Aotus azarae* is unique among
136 the genus because of its cathemeral habits. The Azara's owl monkeys show activity both during the
137 day and night (Fernandez-Duque et al. 2010), which allows for detailed behavioral data to be collected
138 from identified individuals during daytime. The relatively small home ranges (mean \pm SD: 6.2 ha \pm
139 1.8; Wartmann et al. 2014) make it possible to monitor regularly many focal groups (10-15 groups for
140 behavioral data: Fernandez-Duque 2016).

141 *Data collection*

142 We used data collected through two different procedures (demographic monitoring and
143 captures with physical exams). All data were extracted from the Owl Monkey Project (OMP)
144 relational Access database. For demographic monitoring, every time a group of monkeys, or a solitary
145 individual, is found, observers enter an "Avistaje" (i.e., a sighting) in the OMP database, where basic
146 demographic data, geolocation and behaviors noted upon encounter are recorded. We analyzed all

147 avistaje records for which data on hoot calls were available and we analyzed all hoot calls registered
148 during 2001-2017. We analyzed all vocal behaviors entered together with sighting data, not when
149 conducting behavioral focal sampling. To ensure the quality of vocal data, we limited the use of data
150 to those collected by experienced observers, defined as those who stayed in the field at least four
151 months and had experience identifying hoot calls. There were 90 sighting records that included
152 information on hoot calls. Fifty four percent of them (N=48) specified the sex of the caller. For all
153 analyses we classified pair-living adults and floaters as potentially reproductive individuals, and
154 subadults and juveniles still living in their natal groups as non-reproductive ones. We followed Huck
155 et al. (2011) for age classifications of subadults (24.1-48 months) and juveniles (6.1-24 months).

156 Since 2000, we have conducted 277 captures with physical exams of owl monkeys. In the
157 process, we caught 177 different individuals, whom we physically examined, sampled for biological
158 specimens and fitted with a bead or radio collar (Fernandez-Duque and Rotundo 2003; Fernandez-
159 Duque et al. 2017). Given the remarkable lack of visible sexual dimorphism (Fernandez-Duque 2011)
160 these procedures have made it possible to reliably identify individuals in the field. Out of 277
161 captures, we captured potentially reproductive individuals on 191 occasions (69%) and non-
162 reproductive ones on 86 occasions (31%). No hoots were ever registered during captures of non-
163 reproductive individuals. During 29 captures of pair-living adults (15%) there were hoot calls emitted
164 by members of the group left behind.

165 During six of the captures of pair-living adults we obtained sound recordings of hoot vocalizations
166 produced by the remaining adult in the group while the captured pair mate was being examined. We
167 made the recordings with a Marantz PMD660 Recorder and a Sennheiser ME66 Short Shotgun
168 Capsule Head for K6 Series, at a visually estimated distance of 10-30m. All recordings were made by
169 two experienced observers (C. Depeine and M. Rotundo). All audio files (n = 111) were recorded in.

170 wav format, with a sampling rate of 44.1 kHz and 16-bit sound resolution. All recordings were made
171 between 0730 and 1100 hs. The material available was obtained from three males and three females
172 (M1 = 6 calls; M2 = 32 calls; M3= 37 calls; F1 = 8 calls; F2 = 15 calls; F3 = 13 calls).

173 *Acoustic analyses*

174 We performed analyses of the audio files by analyzing calls and syllables separately. To analyze
175 calls, we generated spectrograms of them with a Fast-Fourier Transform using the Audacity (v. 2.2.1)
176 acoustic software set with a Hanning window and a 2048 points window size (gain = 30 dB, range: 40
177 dB). With a 44.1 kHz sampling rate, each sound sample was 22.7 μ s in duration. For acoustic parameter
178 measurements, spectrograms were displayed in the 0–1000 Hz frequency range. The best quality
179 recordings were retained for call analysis (n = 98; 88%) and we used sound samples as a unit to
180 accurately measure the parameters call duration, syllable duration, inter-syllabic-intervals and call rate.

181 To assess sex differences in the acoustic structure of the hoots, we analyzed 69 bisyllabic calls, 30
182 from females (F1= 7 calls; F2= 11 calls; F3= 12 calls) and 39 from males (M1=2 calls; M2= 9 calls;
183 M3= 28 calls). We next analyzed 94 syllables, 42 from females (F1= 14 syllables; F2=6 syllables; F3=
184 22 syllables) and 52 from males (M1= 4 syllables; M2= 12 syllables; M3= 36 syllables). The syllables
185 extracted from each call were labeled according to their position (1 or 2) in the call. To ensure that the
186 loudest peak in each file was the same, all sound files were rectified for DC-offset and normalized
187 using SoundExchange (SoX, v. 14.4.1). Individual syllables were exported in wav format for
188 subsequent analysis with the seewave Package, v. 2.0.5 (Sueur et al. 2008). Many recordings
189 (22/69=32%) were contaminated by low-frequency (<100 Hz) sounds due to equipment handling
190 and/or high-frequency (> 800 Hz) sounds, mainly from insects and birds, that overlapped with the
191 signal of interest. Therefore, using the ‘ffilter’ function of the seewave package, syllables were band-
192 filtered between 100 and 800 Hz where most of the syllable sound energy was concentrated.

193 Subsequent visual inspection of the spectrograms ensured that the signal of interest was devoid of any
194 obvious sound contamination.

195 To quantify the degree of syllable noisiness, we measured the Shannon spectral entropy using the
196 ‘sh’ function of the seewave package. On a scale from 0 to 1, noisy sounds have high entropy compared to
197 purer sounds. To determine the dominant frequency of the signal, we generated a power spectrum of each
198 syllable using the seewave function ‘spec’ and we elected a window length of 4096 points to increase the
199 frequency resolution (10.8 Hz). Using the ‘fpeaks’ function of the seewave package we then searched for
200 the 10 largest peaks of the frequency spectrum. The largest peak of each spectrum was retained as the
201 dominant frequency. In females, the dominant frequency of multisyllabic calls may go up or down and the
202 change is quite noticeable when listening to the audio files. To determine whether there was a consistent
203 pattern in frequency change, we tracked the pitch of 36 tonal hoot syllables using the Praat software (v.
204 6.0.52). To improve pitch accuracy, such analysis was done after reducing the background noise on the
205 spectrogram using the ‘noise reduction’ function in Audacity. Tracking the pitch of graff hoot syllables, on
206 the other hand, was not possible due to their noisy content with many tightly packed bands of modulated
207 sound energy covering a broader frequency spectrum.

208 We automatized all measurements of spectral entropy, syllable duration and dominant frequency
209 together with syllable filtering and graphic displays of frequency spectra with a script written in the R
210 environment (v.3.3.3) (R Core Team 2017).

211 *Statistical Analyses*

212 In considering the data obtained through demographic monitoring, we used a χ^2 test to estimate
213 the probability of obtaining the observed difference in the frequencies of tonal and graff hoots emitted
214 by females and males, or a more extreme one, assuming equal proportions for both sexes as the null

215 model. Additionally, to evaluate the prediction that hoot calls would be female-biased, we used a χ^2
216 test to estimate the probability of obtaining the observed difference in the frequencies of loud calls
217 given by females and males, or a more extreme one, given the null model.

218 All statistical analyses were performed in R version 3.6.0. (R Core Team 2019). We used a linear
219 mixed model using R package lme4 (v. 1.1-21; Bates et al. 2015) to evaluate how well sex predicts the
220 variation of each parameter mentioned above. Due to the extent of non-independence of calls we
221 included the identity of the monkeys as a random factor while sex was fitted as a fixed factor.

222 For syllable analyses we used a linear mixed model to evaluate how well sex and, in this case, the
223 position of syllables in call sequence (syllable one or two) explain the variation on each parameter. We
224 included the identity of the individuals as a random factor and sex and syllable's position as fixed
225 ones. To determine the statistical significance of the models we only compared nested models, varying
226 only in one factor in each comparison. Since random effects were the same in each model, we used the
227 “anova” function (likelihood ratio test) of ‘stats’ package version 3.7.0 in all comparisons.

228 To estimate the probability of obtaining the observed difference, or a more extreme one, in the pitch
229 of female hoot syllable we performed a Wilcoxon test for paired samples. The significant level was set to
230 5% in all analyses and the data are presented as means \pm SD. We also report confidence intervals since they
231 are more informative than p-values alone (Wasserstein and Lazar 2016; Nakagawa and Cuthill 2007; Ziliak
232 and McCloskey 2008). We tested the assumptions of the linear mixed model using visual diagnostic plots
233 for the residuals. None of our parameters violated the assumptions of normality or homogeneity of variance
234 of the residuals.

235 *Ethical note*

236 We have captured individuals regularly since 2000 using an injection rifle and disposable darts
237 loaded with 0.5 ml of ketamine hydrochloride. Since then, we have evaluated the potential effects of
238 capture on animal welfare and on population structure (Juarez et al. 2011; Fernandez-Duque et al.
239 2017).

240 We fitted all individuals with a radio or a bead collar (Fernandez Duque and Rotundo 2003;
241 Juarez et al. 2011; Fernandez Duque et al. 2017), depending on the age of the monkey and our interest
242 in being able to locate it reliably. The radio collar has consisted of a transmitter package mounted on a
243 ball-chain collar with a 15-cm whip antenna.

244 All procedures conformed to Argentinean laws and were approved at different times by the
245 National Wildlife Directorate in Argentina and by the animal research committees of the Zoological
246 Society of San Diego (2000-2005), the University of Pennsylvania (2006-2011) and Yale University
247 (2012-2018). All animal procedures followed the guidelines for the treatment of animals for teaching
248 and research recommended by the Animal Behaviour Society (2014).

249 **Results**

250 Owl monkeys produced two perceptually distinct types of hoot calls (see Online Resources 1
251 and 2 for an audio of tonal and graff hoots). Tonal hoot calls exhibit a narrow-band tonal structure
252 with occasional harmonics whereas graff hoot calls are essentially noisy and broadband, with sound
253 energy appearing fuzzier on the spectrogram (Figure 1). Each type of vocalization can be emitted as a
254 single syllable or as a sequence of two or three similar ones (multisyllabic calls). Here, a syllable is
255 defined as a continuous trace on the spectrogram (Figure 2).

256 Further, males and females emitted different types of vocalizations. **No graff hoots were**
257 **registered from females, nor were any tonal hoots registered from males.** When examining demographic

258 monitoring data, of all records of vocalizations from identified males (N=16), 88% were graff hoots and
259 12% were only classified as “hoot” without specifying which type. On the other hand, for all records of
260 vocalizations from identified females (N=32), 85% were tonal hoots and 15% were vocalizations only
261 described as “hoots”. Females called more often than males (female, N=32; male, N=16 hoots; Chi-
262 square test for given probabilities, $\chi^2=5.33$, $df = 1$, $p=0.02$).

263 Likewise, the capturing of males or females elicited very different responses from the animals
264 left behind. When we captured the adult female in the group only graff hoots were produced by a
265 remaining member of the group (N=16 captures). On 14 of the 16 captures (N=12 different pairs) it was
266 the adult male who produced graff hoots. On the other hand, when we captured the adult male in the
267 group, only tonal hoots were emitted by a remaining member of the group (N=10 captures). In eight of
268 the 10 captures (N=7 different pairs) it was the adult female who emitted the tonal hoots. As noted, in
269 four captures we could not determine the sex or age of the producer of the calls, however, we never
270 heard a tonal hoot during the capture of an adult female or a graff hoot during the capture of a male.
271 Furthermore, we have never registered any hoot, graff or tonal, emitted by a remaining member of the
272 group during 86 captures of non-reproductive individuals (Chi-square test of independence: $\chi^2=112.73$, $df =$
273 1 , $p<0.001$).

274 Calls of males were, on average, shorter than those of females (879 ± 122 vs 981 ± 149 ms
275 respectively). Further, the intersyllable intervals of males were, on average, shorter than those of females
276 (503 ± 105 vs 527 ± 125 ms respectively). The models assessing how well sex of the caller explains
277 duration and intersyllable intervals did not reach statistical significance (Table 1).

278 When analyzing sex differences in the parameters extracted from syllables no model reached
279 statistical significance for explaining statistical variability of duration by the sex of the caller or the
280 position of the syllable. On the other hand, the models did reach statistical significance for explaining

281 variability of entropy and dominant frequency by sex and syllable position (Table 2). Specifically, the
282 entropy was higher in male than female calls and was higher on the first syllable of the hoots analyzed
283 (Table 3). The percentage of variance explained (R^2) by the fixed effects of the model was 48% while
284 the percentage of variance explained by the random effects (individual) was 8%.

285 Similarly, the dominant frequency was higher in male calls, and the second syllable of hoots
286 showed higher values of the variable (Table 3, Figure 3). In this case, the random effects did not explain
287 any variance of the response variable, suggesting no inter-individual variability on dominant
288 frequencies. The fixed effect explained the 28% of model's variance.

289 The pitch of 36 tonal hoot syllables analyzed did not show statistical differences between
290 syllables in any female (Table 4, Figure 4).

291

292 **Discussion**

293 Our study of Azara's owl monkeys provides evidence of sex differences in vocal
294 communication, specifically in loud calls. Detailed registers from identified individuals indicated that
295 females only emit tonal and males only emit gruff hoots. Our data also showed that male calls had
296 higher entropy and dominant frequencies and these parameters also varied depending on the syllable
297 position (one or two). Further, sex and syllable position explained more of the variation of entropy
298 than the variation of dominant frequency

299 The quantitative analyses of owl monkey hoots indicate that there are sex-related differences
300 in their calls. Given the fact that owl monkeys from Argentina do not exhibit any striking
301 morphological/anatomical differences between sexes (Fernandez Duque 2011), it is likely that such
302 differences arise from allometric traits driven by factors other than body size or body weight (Garcia et

303 al. 2017). In the past, it has been proposed that sexual selection may have played a role on the
304 evolution of sexual dimorphism in loud calls (Delgado 2006; Snowdon 2017). The sexual dimorphism
305 we found in *Aotus* calls, has been proposed for other non-sexually dimorphic primate taxa as in indris
306 (*Indri indi*, Gamba et al. 2016), golden lion tamarins (*Leontopithecus rosalia*, Benz et al. 1990),
307 common marmosets (*Callithrix jacchus*, Norcross and Newman 1993) and Wied's black-tufted-ear
308 marmosets (*Callithrix kuhlii*, Smith et al. 2009). A possible explanation could be that differences in
309 male and female calls allow other individuals to identify the sex of the caller; however, a more definite
310 evaluation of this hypothesis requires an approach that includes certain manipulation of conditions,
311 like playback experiments. From early on in the Owl Monkey Project, we have relied on playing back
312 hoot calls to assess presence/absence of owl monkeys in remote areas and to find groups and floaters
313 for capturing them. In the beginning, we did not have an adequate understanding of their vocal
314 communication. So, it was only retrospectively that we realized that the playing back of gruff hoots
315 was more effective in attracting individuals and usually led to the capture of females. Further,
316 preliminary results from systematic ongoing playback experiments led by one of us (AGC), have
317 shown that male and female pairs reacted differently to gruff and tonal hoots recordings, with both
318 sexes showing an increase of socio-sexual behaviors toward their partners when a simulated same sex
319 competitor was played back (García de la Chica, unpublished). Although beyond the scope of this
320 study, these preliminary findings suggest that some acoustic parameters in owl monkey hoot calls
321 allow individuals for identification of the sex of the caller.

322 It follows that our data seem to support the hypothesis proposed by Heymann (2003) that in
323 taxa where males are the principal providers of infant care, loud calls would be female-biased. Given
324 that in monogamous species, sexual selection may apply equally to both sexes, and that in owl
325 monkeys, males are heavily involved in energetically costly parental care activities (Rotundo et al.

2005; Huck and Fernandez Duque 2012) it may be that females face greater intrasexual competition to choose the best male. Our data suggested a female-biased pattern in loud calls with females vocalizing up to twice as much as males. This female-biased pattern in the production of loud calls show contradictory results in the literature. While in captive common marmosets (*Callithrix jacchus*) and golden lion tamarins (*Leontopithecus rosalia*) males and females showed similar rates of loud calls (McLanahan and Green 1977; Norcross & Newman, 1993), results from captive *Saguinus oedipus* showed adult females emitting almost three times more loud calls than males (McConnell and Snowdon 1986).

Unlike the cooperative breeding social systems usually observed in callitrichid primates, *Aotus* groups do not contain “helpers” and infant care is provided exclusively by the adults in the group (Rotundo et al. 2005; Huck and Fernandez-Duque 2012). Infants are transported by the adult male 84% of the time after the infant’s first week of life (Rotundo et al. 2002); even during the exceptional cases of twin births, which could entail a double effort of transporting the infants, the mothers did not carry them more frequently than mothers of singletons (Huck et al. 2014b). These observations, even from relatively infrequent events, may be indicating that there are selective pressures against *Aotus* females to increase the amount of infant care.

Our findings notwithstanding, like for most of field primatology data ever collected, one must be cautious and consider other factors that may be influencing observational data. First, with only six individuals included in our acoustical analyses the sample size is small, and the contribution of each individual on the analyses is not the same, with a big variability especially for males. Thus, even when this variability should be partially controlled by the design of our statistical models, our results must be interpreted with caution and data from more identified individuals are undoubtedly needed for further comparisons. Further, we cannot rule out the influence of potential observer bias in noting and

349 recognizing tonal hoots more frequently than graff ones or the fact that it is impossible to have a true
350 systematic random sampling design. To address the latter issue, we are currently implementing the use
351 of passive terrestrial acoustic recorders, which allow true systematic recording across the 24 hours.
352 Unfortunately, we still do not know if loud calls encode signals about pair membership, which could
353 inform other individuals about the social condition of callers without the necessity of direct, costly
354 fighting. More acoustic data on identified pairs and solitary individuals will show if the structure of
355 hoot calls present differences in relation to the social condition of owl monkeys, which will provide us
356 deeper insights into the evolution of vocal mechanism regulating pair bonding and mate choice
357 strategies in this species.

358 **Conflict of Interest:** The authors declare that they have no conflict of interest.

359 **Ethical approval:** All applicable international, national, and/or institutional guidelines for the care
360 and use of animals were followed.

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534

535 **Figure 1**

536 Exemplars of *Aotus* hoot calls for three females and three males. Two variants are shown: A tonal hoot
537 from female 2 (F2) showing the presumptive fundamental sound (f_0) with two overlaid harmonics ($2f_0$
538 and $3f_0$). A graff hoot from male 3 (M3) in which the first syllable is less noisy (lower entropy)
539 compared with the second syllable. The latter displays many bands of sound energy between 200 Hz
540 and 600 Hz (arrows). Note the change in the pitch of the female hoot calls between first and second
541 syllable: the pitch increases (F1), remains stable (F2) or decreases (F3).

542 **Figure 2**

543 Spectrograms of Azara's owl monkeys loud calls: (a) a graff hoot from male 2 and (b) a tonal hoot from
544 female 1. The temporal acoustic parameters measured in this study are shown at bottom. For each type
545 of call, a power spectrum of the second syllable is shown at right. The vertical arrowhead points to the
546 syllable dominant frequency (see text for more details). dur: call duration; s1: duration of syllable 1; s2:
547 duration of syllable 2; isi: intersyllable interval; oto: onset-to-onset time interval.

548 **Figure 3**

549 Effect plot of entropy (a) and dominant frequency (b). On both graphics, sex differences (F=Females
550 and M=Males) are showed on the left while syllable differences (1=first syllable of the calls and
551 2=second syllable of the calls) are showed on the right

552 **Figure 4**

553 Pitch analysis of a hoot call (female 1, call 2). The top panel of the Praat software window shows the
554 amplitude modulation of the call. The bottom panel shows the spectrogram, which displays both
555 frequency values (in red) and pitch values (in blue). After background noise reduction, the blue line
556 falls exactly on the presumptive fundamental of S1 and S2, respectively. The two vertical red lines
557 denote the time interval selected to collect a vector of pitch values, which was saved as a text file

558

559 **Table 1**

560 Result of model comparisons among models including single fixed effects (sex) and the null model.

Dependent variable	Model	χ^2	df	P
Duration (ms)	Model 1 (Sex), Null	0.25	1	0.62
Intersyllable interval (ms)	Model 1 (Sex), Null	0.02	1	0.88

561

562 **Table 2**
 563 Result of model comparisons among complete models (including the interaction among predictor
 564 variables sex and syllable), models including simple fixed effects (sex and syllable) and models
 565 including single fixed effect. When models with only one fixed variable did not account for enough
 566 variance to reject the null hypothesis at the specified significance level, we compared the simplest
 567 model with the null one.

Dependent variable	Model	χ^2	<i>df</i>	P
Duration (ms)	Complete Model (Sex*Syl), Model 1 (Sex+Syl)	0.84	1	0.34
	Model 1 (Sex+Syl), Model 2 (Sex)	0.16	1	0.7
	Model 2 (Sex), Null	0.09	1	0.8
Entropy (Hz)	Complete Model (Sex*Syl), Model 1 (Sex+Syl)	0.44	1	0.5
	Model 1 (Sex+Syl), Model 2 (Sex)	6.05	1	0.01
	Model 1 (Sex+Syl), Model 3 (Syl)	11.71	1	<0.01
Dominant Freq (Hz)	Complete Model (Sex*Syl), Model 1 (Sex+Syl)	0.21	1	0.6
	Model 1 (Sex+Syl), Model 2 (Sex)	5.3	1	0.02
	Model 1 (Sex+Syl), Model 3 (Syl)	14.1	1	<0.01

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581 **Table 3**

582 Sex differences in the mean, SD and confident intervals (95%) of the statistically significant variables
 583 (entropy and dominant frequency) extracted from syllable analyses.

584

Variable		Mean	SD	Confidence interval (95%)	
				Lower	Upper
Entropy	Females	0.47	0.04	0.46	0.48
	Males	0.52	0.02	0.51	0.52
	Syllable 1	0.50	0.03	0.49	0.51
	Syllable 2	0.49	0.04	0.48	0.50
Dominant Frequency	Females	0.31	0.03	0.30	0.32
	Males	0.34	0.03	0.33	0.35
	Syllable 1	0.32	0.03	0.31	0.33
	Syllable 2	0.33	0.03	0.32	0.34

585

586 **Table 4.**

587 The pitch value (mean \pm SD) of female hoot syllables is shown according to syllable position (S1 vs. S2) within
 588 the call. The p-values associated with the paired samples Wilcoxon statistics (V) are reported separately for
 589 each female. n = number of calls.

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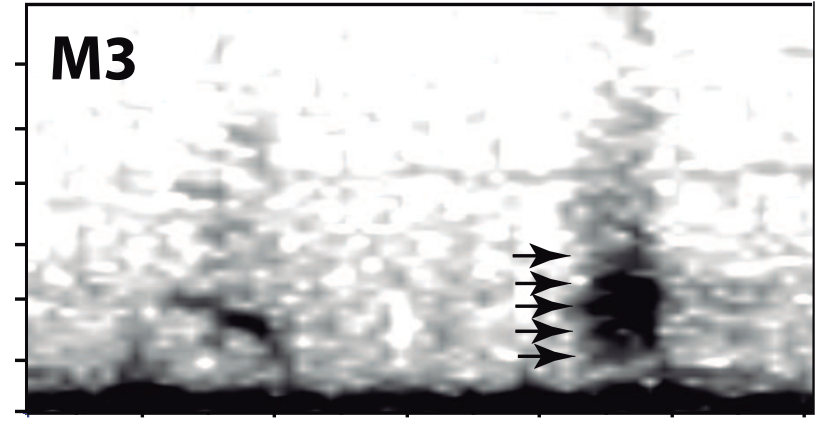
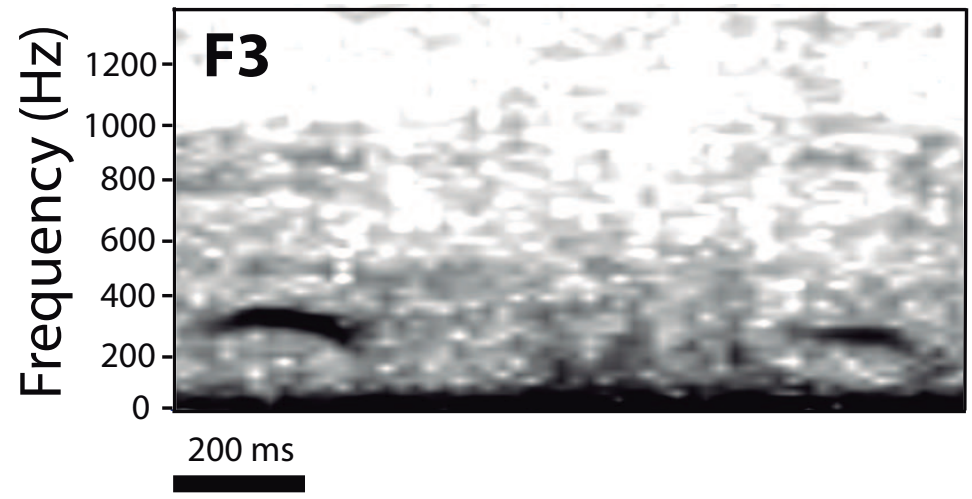
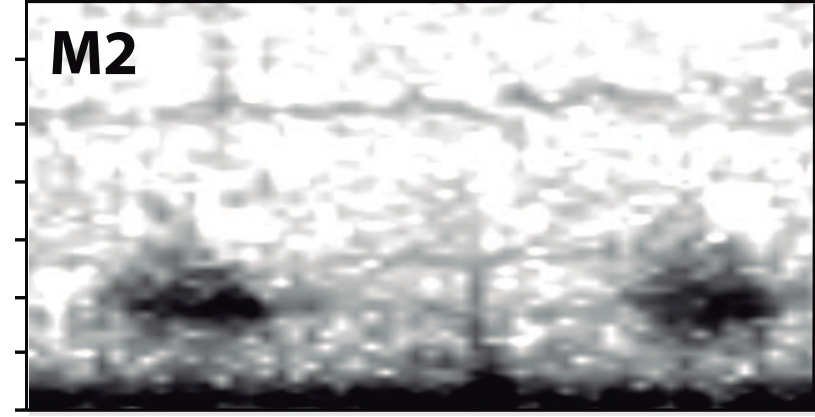
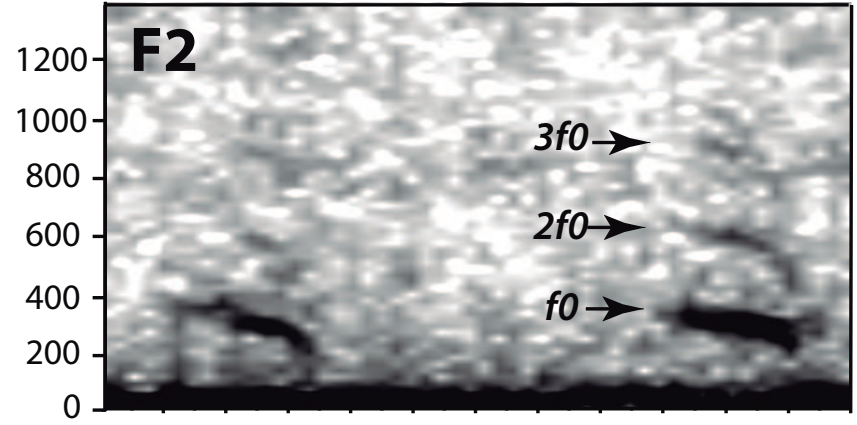
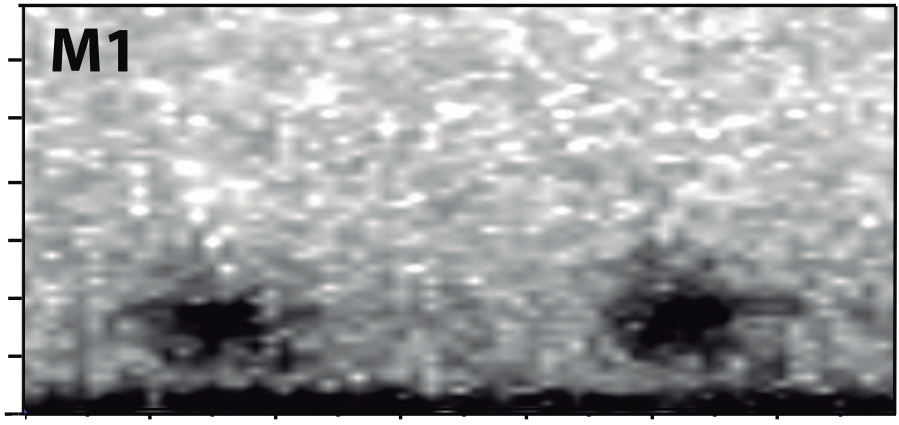
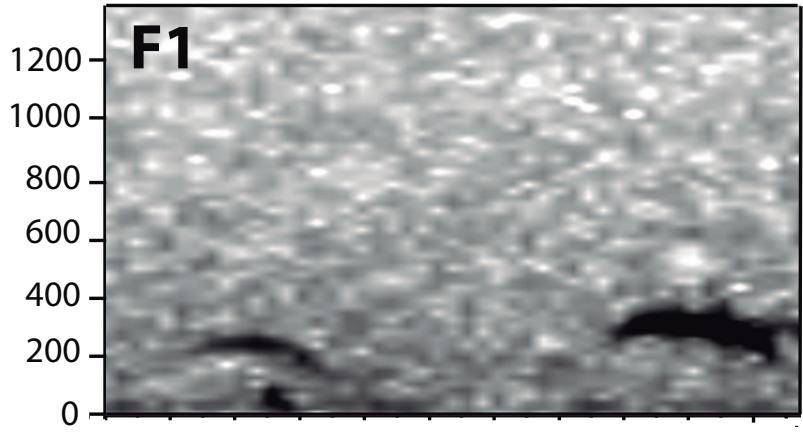
Female	n	S1	S2	V	p-value
F1	6	292.40 \pm 14.44 Hz	314.02 \pm 31.00 Hz	2	0.0938
F2	6	309.52 \pm 31.76 Hz	292.19 \pm 9.54 Hz	16	0.3125
F3	6	300.23 \pm 9.67 Hz	295.90 \pm 22.61 Hz	28	1.0000

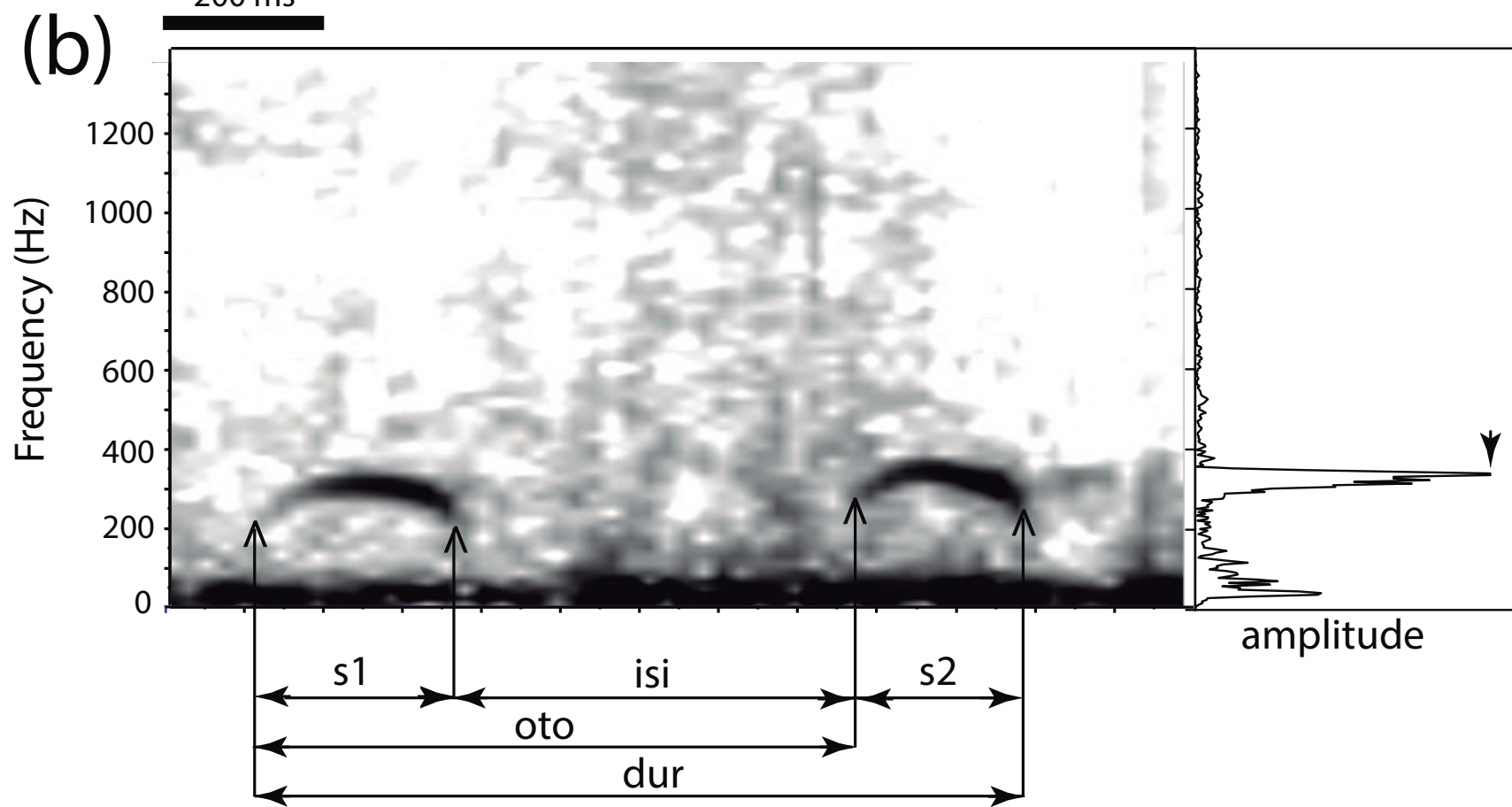
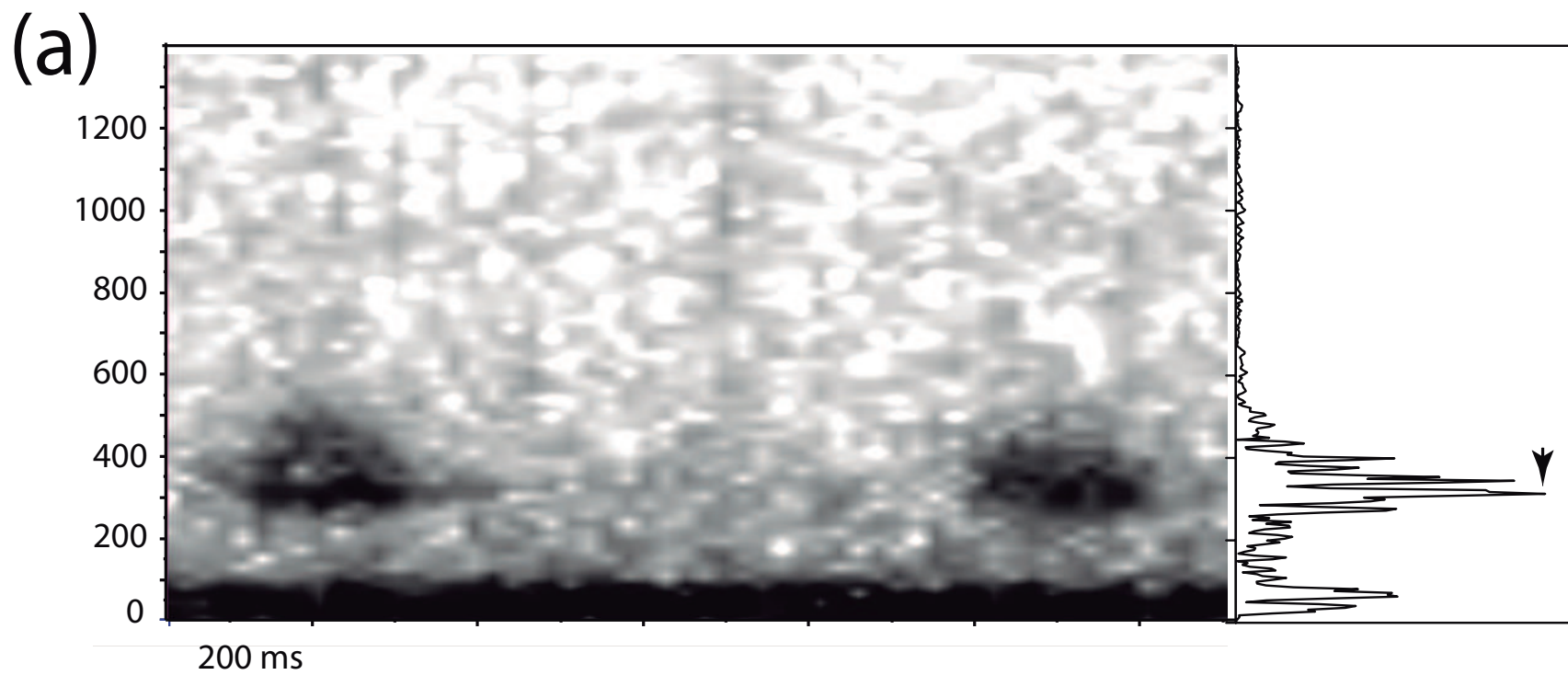
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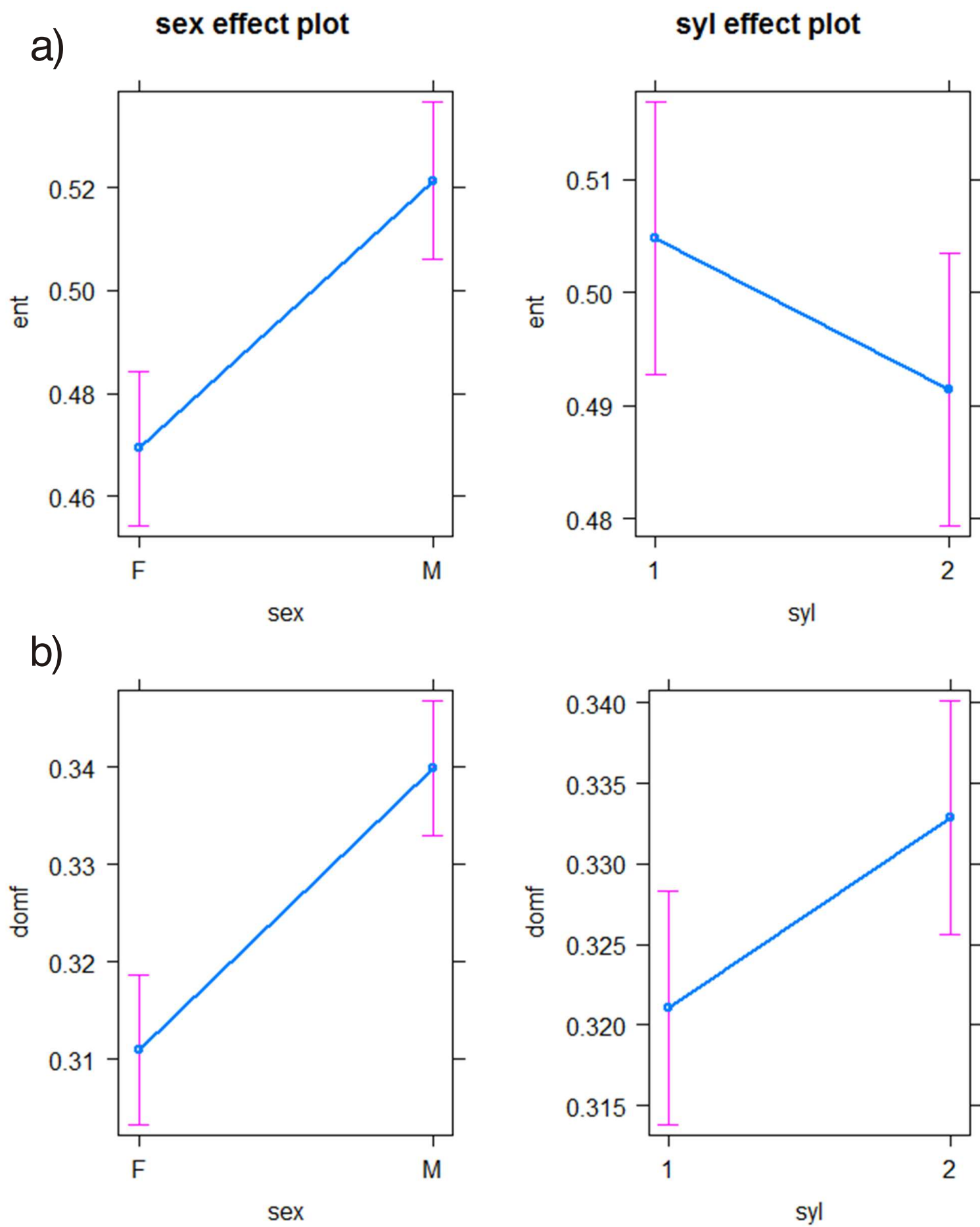
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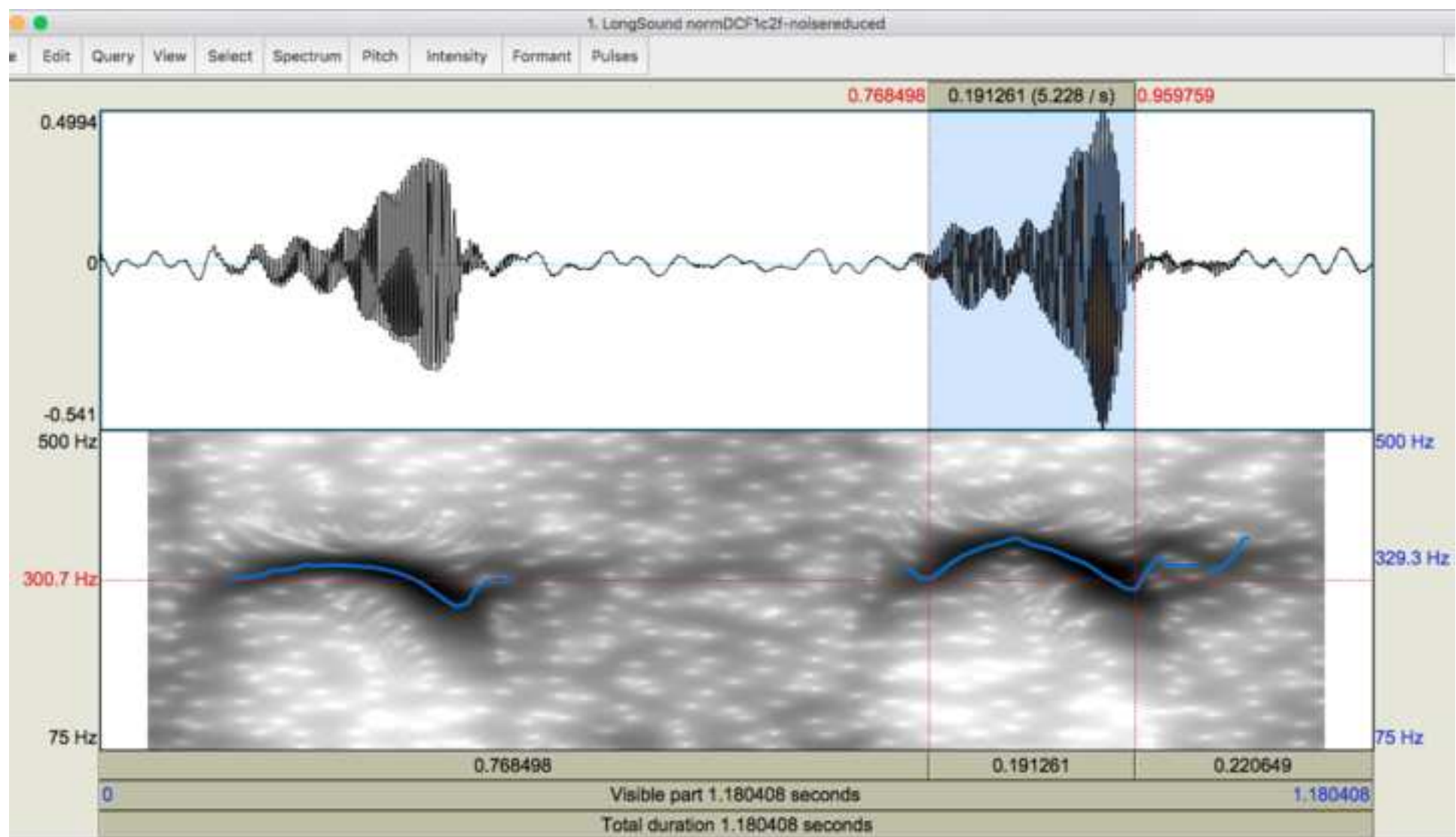
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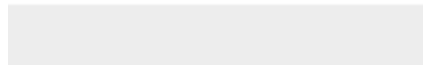






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