

1 **Pied tamarins change their vocal behavior in response to noise levels in**
2 **the largest city in the Amazon**

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30 **Short title:** Pied tamarin responses to anthropogenic noise

31 **Abstract**

32 Many animal species depend on sound to communicate with conspecifics. However,
33 human-generated (anthropogenic) noise may mask acoustic signals and so disrupt
34 behavior. Animals may use various strategies to circumvent this, including shifts in the
35 timing of vocal activity and changes to the acoustic parameters of their calls. We tested
36 whether pied tamarins (*Saguinus bicolor*) adjust their vocal behavior in response to city
37 noise. We predicted that both the probability of occurrence and the number of long calls
38 would increase in response to anthropogenic noise and that pied tamarins would temporally
39 shift their vocal activity to avoid noisier periods. At a finer scale, we anticipated that the
40 temporal parameters of tamarin calls (e.g., call duration and syllable repetition rate) would
41 increase with noise amplitude. We collected information on the acoustic environment and
42 the emission of long calls in nine wild pied tamarin groups in Manaus, Brazil. We found that
43 the probability of long-call occurrence increased with higher levels of anthropogenic noise,
44 though the number of long calls did not. The number of long calls was related to the time of
45 day and the distance from home range borders - a proxy for the distance to neighboring
46 groups. Neither long-call occurrence nor call rate was related to noise levels at different
47 times of day. We found that pied tamarins decreased their syllable repetition rate in
48 response to anthropogenic noise. Long calls are important for group cohesion and
49 intergroup communication. Thus, it is possible that the tamarins emit one long call with
50 lower syllable repetition, which might facilitate signal reception. The occurrence and
51 quantity of pied tamarin' long calls, as well as their acoustic proprieties, seem to be
52 governed by anthropogenic noise, time of the day and social mechanisms such as proximity
53 to neighbouring groups.

54

55 **Keywords:** sound pollution, soundscape, urban ecology, primates, Amazon, *Saguinus*
56 *bicolor*

57 Abbreviations

58 IUCN International Union for conservation of Nature

59 INMET Brazilian Institute for Meteorology

60 HR Home Range

61 DFT Discrete Fourier Transform

62 SISBIO/MMA (System in Biodiversity/ Ministry of Environment)

63

64 **Introduction**

65 Many animal species, especially those that live in social groups, depend on sound to
66 communicate with conspecifics. Vocal communication is used to transmit information
67 concerning essential social behaviors, such as cooperatively foraging for high quality food
68 resources (Caine et al., 1995), defense against predators (Kirchhof & Hammerschmidt,
69 2006), territorial activities (Sobroza, Gordo, Barnett, et al., 2021), group cohesion (Kondo &
70 Watanabe, 2009), assessment of competition (Benítez et al., 2017), and reproduction
71 (Duarte et al., 2011). All these activities can be disrupted in noisy environments, via the
72 masking of acoustic signals and/or the distraction of producers and receivers (Aaden et al.,
73 2011; Allen et al., 2021; Brumm & Slabbekoorn, 2005; Buxton et al., 2020; Grabarczyk &
74 Gill, 2019; Huet des Aunay et al., 2017). In recent decades, anthropogenic noise has
75 increased worldwide, imposing new selective pressures on wildlife (Sih et al., 2011;
76 Slabbekoorn et al., 2010; Swaddle et al., 2015). Responses to such disturbances have
77 been reported across ecological scales, from individuals to populations, communities and
78 cross-species interactions (Barbosa et al., 2020; Hubert et al., 2018; Slabbekoorn &
79 Halfwerk, 2009; Soudijn et al., 2020).

80 Animals may use various strategies to avoid or reduce noise impacts (Brumm et al.,
81 2004; Brumm & Slabbekoorn, 2005). Temporal displacement of vocal activity is a possible
82 response to anthropogenic noise. For instance, a variety of bird species are known to shift
83 the timing of their vocal activity from day to night as noise increases (Fuller et al., 2007; Gil
84 et al., 2015). Such species naturally have peaks and troughs of vocal activity throughout the
85 day, which may be related to social behavior as well as optimizing acoustic transmissibility
86 (Pérez-Granados & Schuchmann, 2021; Waser & Brown, 1984). Such vocal activity peaks
87 may also be timed to avoid overlap with periods when other species emit sounds (i.e., the
88 acoustic niche hypothesis; Schneider et al., 2008). However, such diel patterns may also
89 alter due to anthropogenic noise (Sierro et al., 2017).

90 At a finer scale, some species also change the acoustic parameters of their calls,
91 including frequency, time and amplitude (Brumm & Slabbekoorn, 2005). Doing this can
92 potentially avoid obscuring spectral features and so enhance signal-to-noise ratio and
93 signal audibility (Brumm & Slabbekoorn, 2005; Slabbekoorn & Peet, 2003). It has been
94 suggested that some species may emit higher pitched calls to avoid spectral overlap with
95 anthropogenic noise (Brumm & Bee, 2016; Roca et al., 2016; Slabbekoorn & Peet, 2003),
96 as this often (though not always) has more energy at lower frequencies (Alquezar &
97 Macedo, 2019; Caorsi et al., 2019; Gill et al., 2015). Such trends may be reversed when
98 sound levels are extremely loud, and animal hearing is impaired (Wolfenden et al., 2019). In
99 such cases, some animals, such as birds, find it difficult to detect higher frequencies and so
100 may stop producing them largely or entirely; consequently, their calls become dominated by
101 lower frequencies (Wolfenden et al., 2019). When facing intense noises, some fish and
102 primate species also increase call duration and rate to increase detectability by the receptor
103 (Brumm et al., 2004; Egnor et al., 2007; Picciulin et al., 2012; Santos et al., 2017). In
104 contrast, other studies show that individuals cease calling when they are unlikely to be

105 heard, presumably as a means of minimizing energy expenditure (Duarte et al., 2019;
106 Kaiser et al., 2011).

107 Primates are ecologically important in tropical forests, especially because of their
108 roles in seed dispersal and associated ecological processes such as forest regeneration
109 and carbon storage (Fuzessy et al., 2016, 2021; Heymann et al., 2019; Peres et al., 2016).
110 However, many primates are also threatened by urbanization and its consequences, such as
111 the risk of being run over by cars, attacked by domestic animals, electrocuted by power-
112 lines, and by increased risks of disease transmission (Thatcher et al., 2023). Additional,
113 indirect, threats, such as that of anthropogenic noise, may also play an important role in
114 their daily lives. For instance, Black-tufted marmosets (*Callithrix penicillata*) prefer to use
115 silent areas of their home ranges, even if they contain fewer food resources (Duarte et al.,
116 2011). Black-fronted titi monkeys (*Callicebus nigrifrons*) residing in noisy areas near active
117 mines, often reduce and change their daily patterns of vocal activity (Duarte et al., 2018). In
118 contrast, Bolivian grey titi monkeys (*Plecturocebus donacophilus*) appear to be resilient to
119 aural disturbance, and do not change their general activity budget or movement patterns in
120 response to anthropogenic noise (Linerós et al. 2020). Therefore, primate behavioral
121 responses towards anthropogenic noise appear to be predominantly species-specific.

122 Many primate species produce long distance calls (often termed “long calls”), that
123 are usually high amplitude and often have multiple functions, including maintenance of
124 group cohesion, delimiting territories, notification of food availability and mate defense
125 (Dolotovskaya & Heymann, 2022; Hopkins, 2013; Snowdon, 2017; Wich & Nunn, 2002).
126 For example, in southern brown howler monkeys (*Alouatta guariba*) vocalizations play an
127 important role in intergroup competition, and long call emission tends to be spatially
128 structured, with animals roaring more often at territorial boundaries (Da Cunha & Jalles-

129 Filho, 2007). However, long calls may also be important during intergroup encounters, as
130 they can provide information on numerical odds (Kitchen, 2004).

131 Callitrichids are known for their intense vocal activity (Snowdon, 2001), they typically
132 call when further apart (>15 m), but vocalization is also linked with interactions with extra-
133 group individuals and intergroup encounters (Caselli et al., 2018; Snowdon, 2001).
134 Nevertheless, such activity is also temporally structured, with animals calling more in the
135 morning (Heymann, 1990; Norconk, 1990). Despite the importance of long calls to primate
136 species, our knowledge of how, in the wild, their deployment is adjusted in response to
137 anthropogenic noise, is limited (Duarte et al., 2011, 2018; Gómez-Espinosa et al., 2022;
138 Lineros et al., 2020; Santos et al., 2017).

139 Pied tamarins (*Saguinus bicolor*) are Amazonian callitrichids with a highly restricted
140 geographic range encompassing a total of just 7,500 km², much of which occurs in urban
141 and peri-urban areas of Manaus, the largest city in the Brazilian Amazon (Gordo et al.,
142 2013, 2017). Urbanization has restricted individual groups to isolated forest fragments, and
143 these are surrounded by a noisy urban matrix (Gordo et al., 2013). Mainly due to
144 fragmentation and its consequences (e.g., road-kill, electrocution, and attacks from
145 domestic animals), the species is considered Critically Endangered by the IUCN (Gordo et
146 al., 2019). The species has 12 identified call types in its vocal repertoire, which includes
147 long calls (Sobroza et al. 2017). Pied tamarin long calls are signals composed of variable
148 number of syllables (not more than 8) with a \cap -shaped spectral signature and relatively
149 constant interval between syllables (Sobroza, Gordo, Pequeno, Dunn, et al., 2021a). They
150 are emitted during inter- and intra-group interactions, such as territorial defense, group
151 movement and cohesion (Sobroza et al., 2017; Sobroza, Gordo, Pequeno, Dunn, et al.,
152 2021a). While noise levels in captivity are known to have a low impact on pied tamarins

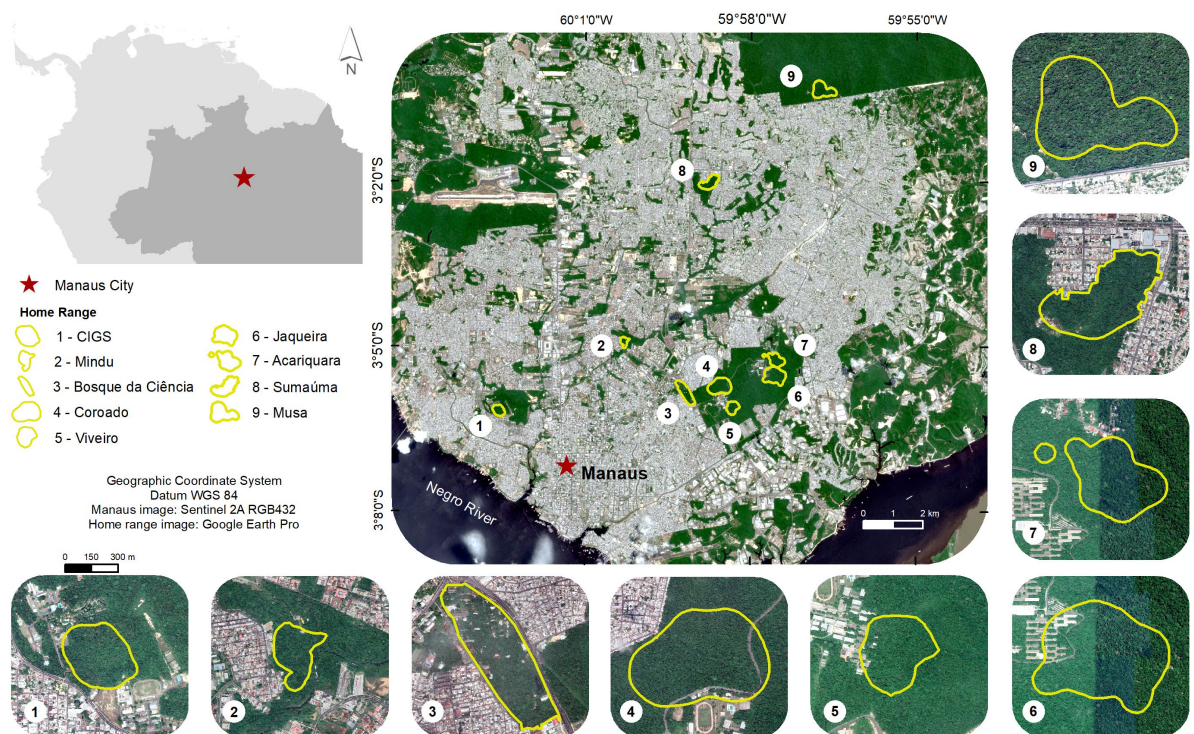
153 feeding and vigilance behavior (Steinbrecher et al. 2023), little is known about the impact of
154 noise on pied tamarin vocal behavior in the wild.

155 The current study aimed to evaluate whether pied tamarins alter their communication
156 behavior in response to urban noise. For this, we followed nine wild pied tamarin groups
157 from urban and peri-urban areas and assessed whether their vocal behavior was related to
158 variation in anthropogenic noise (Brumm & Slater, 2006; Deoniziak & Osiejuk, 2016). When
159 faced with high intensity noises, some animals cease calling (Vargas-Salinas & Amézquita,
160 2014). Therefore, we analysed both long call occurrence, i.e. decisions of individual animals
161 to call or not (binary data) and the number of long calls (count data) separately. We
162 predicted that both the occurrence and number of long calls would increase with an
163 increase in anthropogenic noise, such that signal content would become redundant and
164 more likely to reach the receiver (Brumm & Slater, 2006; Deoniziak & Osiejuk, 2016). We
165 also predicted that long call occurrence and the number of long calls would increase in
166 response to increased noise levels. Many primate species have a peak of vocal activity in
167 the morning (do Nascimento et al., 2021; Ravaglia et al., 2023), though, we predicted that
168 pied tamarins would shift such a pattern to avoid the noisiest periods of the day. At a finer
169 scale, we tested whether temporal parameters of the acoustic features of the tamarin long
170 calls (duration and syllable repetition rate) would increase with noise amplitude. At the
171 same time, we predicted that the dominant and lowest frequencies would remain similar, as
172 pied tamarin long calls are high-pitched, ranging from 6 to 9 kHz (Sobroza et al., 2017), and
173 in primates frequency features are expected to be less likely to be modulated than temporal
174 features (Janik & Slater, 1997).

175 **Methods**

176 *Study area*

177 The current study was conducted in the central Brazilian Amazon, in the city of
 178 Manaus, Amazonas State, Brazil (Figure 1). Manaus is an industrial city that has expanded
 179 in a disorderly and poorly-regulated way since the 1970s (Coelho et al., 2018).
 180 Consequently, while the city houses more than 2-million people, it also contains over 900
 181 forest fragments of different shapes and sizes (Coelho et al., 2018). The average
 182 temperature during the sampling period was 27.7 C° (SD= ± 2.6), and average relative air
 183 humidity was 73.27% (SD = ± 13.94) (Brazilian Institute for Meteorology INMET- station
 184 A101). The distance from the sampling areas to the meteorology station varied from 3.04 to
 185 14.71 km (mean = 4.98, SD = ± 4.33). Our study sites comprised five forest fragments
 186 ranging from 24 to ~700 ha managed by different organizations (private, municipal, state,
 187 and federal - including one military area). The fragments have been isolated for between 30
 188 and 68 years (Gontijo, 2008), and each is surrounded by asphalted roads. Nine groups of
 189 pied tamarins were followed in five forest fragments of the city of Manaus and one in a
 190 continuous forest area at the city's periphery (Table 1) (Figure 1).



192 Figure 1. Locations (large maps) and shapes (small maps) of home ranges of pied
193 tamarin (*Saguinus bicolor*) groups (1-9) followed in the urban area of Manaus, Brazil (larger
194 map) from November 2018 to December 2019.

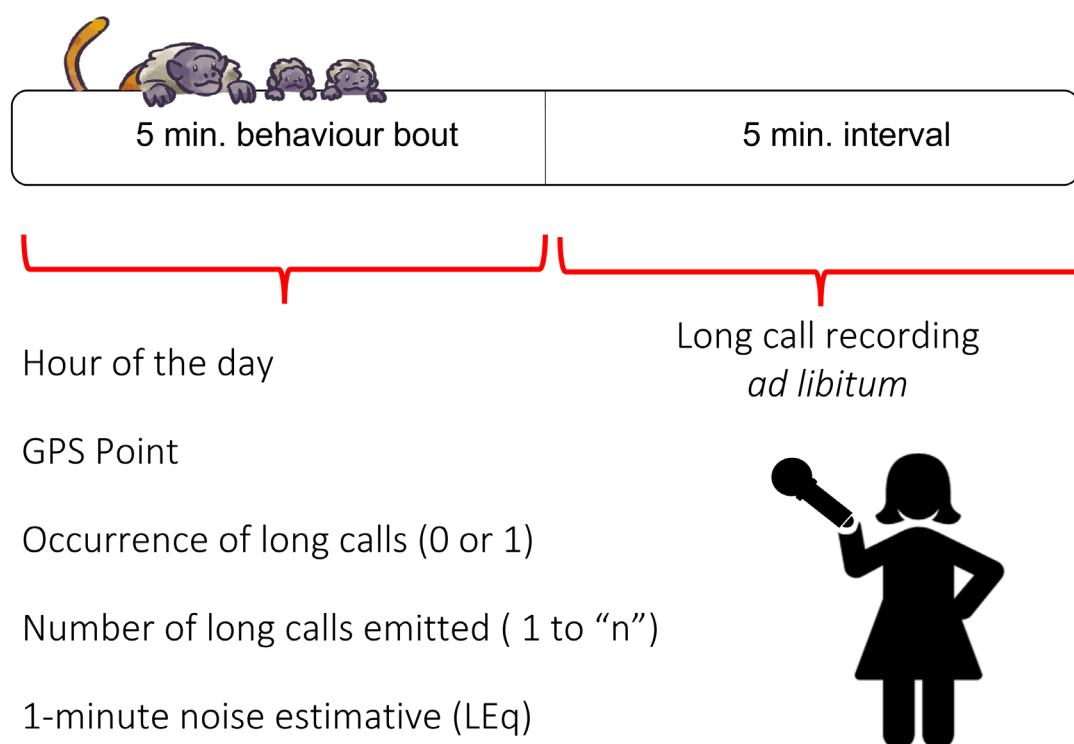
195 *Subject and capture protocols*

196 Data were collected between November 2018 and December 2019. We followed
197 nine pied tamarin groups (Table 1), using radio-telemetry in seven groups to facilitate
198 location and monitoring. The other two groups were well-habituated to humans and so
199 could be followed without radio-telemetry. For those where radio-telemetry was used, we
200 captured whole groups (Lagroteria et al., 2017; Sobroza et al., 2017) and attached a SOM
201 2380 transmitter (Wildlife Materials, US) to the alpha female of each group, as such
202 individuals are highly philopatric (Gordo, unpublished data). Each attached transmitter
203 emitted a different frequency (across a range from 164.00-164.99 MHz), and these were
204 detected with a two (H) or three (Yagi) element antenna and an ATS® receptor (164-168
205 MHz). The success of group detection varied when using two or three-element antenna,
206 which relate to different number of behavioral bouts recorded across groups (Table 1).
207 Groups were captured seven days before data collection for research covering three
208 independent projects: two related to pied tamarin health (Chaves et al., 2022) and the
209 current behavioral study.

210 *Data collection*

211 A researcher and a field assistant followed each group for 10 consecutive days from
212 ~ 06:30-17:00, the main period of pied tamarin activity (Egler, 1986). Pied tamarins sleep
213 relatively early, ~15:30-16:00 (Gordo et al. 2017), possibly as an anti-predatory strategy
214 (Caine, 1987). Thus, when around this time if we noticed that the study group selected a
215 tree and remained in the same place for at least 40 minutes, we stopped the data collection
216 for the day. In the field, we collected vocal data during five-minute behavioral bouts followed

217 by five-minute intervals (Martin & Bateson, 1993). During each such bout we registered
 218 whether long calls occurred or not (one or zero) and aurally quantified the number of long
 219 calls emitted by all group members during the sampling bout. We also recorded the group's
 220 location via GPS (GPSMap 78s) and estimated noise levels (Figure 2).



221

222 Figure 2 Sampling scheme showing the nature of data collected during 5- minute5-
 223 min behavioral bouts and intervals between bouts. Each pied tamarin group was followed
 224 for 10 consecutive days from 6:00 a.m. to ~ 5:00 p.m. We recorded a total of 1953 bouts
 225 from nine pied tamarin groups (Table 1). Within these, 472 bouts had at least one long call.

226

227 During each of these 5-minute sampling bouts, we also took a 1-minute sample of
 228 noise amplitude by calculating the equivalent continuous sound levels (LEeq) with a C
 229 weighting (dbC) that is appropriate for recording low-frequency sound. The LEq is a time-
 230 averaged sound level that, in this survey, was estimated based on sound pressure levels

231 assessed every second during the 1-minute readings of a calibrated CEL-246 sound level
232 meter (Casela Solutions).

233 After the end of the 10 day group-following period, we estimated the home range
234 (HR) boundaries via the sum of all the GPS points recorded. To do so, we used a kernel
235 density estimator with a reference smooth parameter for all groups (Worton, 1989). We
236 defined HR as the area in which 95% of the points were found (Anderson, 1982). To
237 estimate HR limits, we used the *adehabitatHR* package (Calenge, 2018) in R (R
238 Development Core Team 2020). We estimated the HR limits only to assess the distances
239 from each point to the polygon of HR boundaries (a proxy for the distance to neighbouring
240 groups). To estimate such distances, we used the *Near* function from ArcMap 10.5.

241 *Acoustic analysis*

242 During the five-minute intervals between behavioural bouts we recorded pied tamarin
243 long calls *ad libitum* (Figure 2) for subsequent acoustic analysis. Recordings were not made
244 continuously during these five minutes, as we chose the more appropriate moments to
245 record (i.e. shorter distances between the animals and the recorder and lower background
246 noise). We recorded the calls as .wav files using a Sennheiser-ME67 microphone
247 (microphone frequency response: 1-16.0 kHz), attached to a Zoom H4N recorder (16-bit,
248 sampling rate = 44 kHz). Since we did not estimate noise levels during the intervals that we
249 recorded the calls (see Figure 2), we considered the noise levels estimated from the five-
250 minute bout prior to the long calls recorded in the subsequent five-minute interval. We
251 analyzed the long calls using Raven 1.6 (Bioacoustics, 2019). For each call, we analyzed
252 four acoustic parameters: dominant frequency, lowest frequency, duration and syllable
253 repetition rate (number of syllables divided by call duration). Using Raven, we estimated the
254 parameters from the first (fundamental) harmonic, using the *power spectra* tool to select
255 only high-quality sounds and avoid biased estimates (Zollinger et al., 2012) via a -20 dB

256 cut-off relative to the frequency with maximum energy (Podos, 1997). After such filtering,
257 520 calls remained for analysis. We distinguished consecutive long calls based on
258 amplitude and general pattern of increasing syllable duration and clearer \cap -shape definition
259 as the call reached its end. Only non-overlapping long calls were used for acoustic analysis.
260 Spectrograms were built with the following configuration: DFT size = 1024 samples, overlap
261 = 80%, window size = 20 ms, window type = *Blackmann*.

262 *Statistical analysis*

263 To test whether the occurrence of tamarin long calls was related to anthropogenic noise
264 and time of day, we used Generalised Linear Mixed Models (GLMM) (Zuur et al., 2009). In
265 the first analysis, each behavioral bout was the sampling unit (Figure 2), and the
266 occurrence of long calls was the response variable (1 = occurred; 0 = did not occur)
267 (N=1953), assuming binomial errors and a logit link. We used 1-minute sound level
268 estimate and time of day as predictors. Because the occurrence of long calls may be
269 related to other social aspects of intra- and inter-group communication (Snowdon 2001), we
270 also included group size and distance from the border of their home ranges (a proxy for
271 proximity to neighbouring groups) as predictors. We included fragment and group identity
272 as random factors to control for non-independence between observations of the same
273 group, differences in sampling sizes (Table 1), and any fragment-level variation (for
274 instance differences in fragment size and resource availability). Initially, we included an
275 interaction factor between sound level and time of day to evaluate whether tamarins altered
276 their vocal activity throughout the day in response to sound level variation. As this
277 interaction term was statistically non-significant ($p > 0.1$) we removed it and reran the model
278 (Table S1). The procedure of removing interaction terms from linear models is
279 recommended when the interaction is not significant, since misinterpretation of the effect of
280 other variables might otherwise occur (Engqvist, 2005).

281 We also used a GLMM with the same predictors to test whether the number of long calls
282 was related to anthropogenic noise, time of the day, and social aspects (e.g. group size and
283 proximity to neighbouring groups). As before, we initially included an interaction factor
284 between sound level and hour of the day to evaluate whether pied tamarins alter their vocal
285 activity during daylight hours. Once again, the interaction term was not statistically
286 significant ($p > 0.1$), so we removed it and re-ran the model. For this analysis, we used a
287 subset of behavioral bouts that contained at least one long call (N=472) as sampling units.
288 For this model, we used a negative binomial distribution with log link (Zuur et al., 2009)
289 (Table S1).

290 To test whether pied tamarin long call acoustic features changed in response to
291 anthropogenic noise, we used a GLMM for each of the acoustic parameters. In this case,
292 each long call was used as a sampling unit (N=520), and the response variables were
293 dominant frequency, lowest frequency, duration, and syllable repetition rate. For all models,
294 we used the 1-minute sound level as predictor, and fragment and group identity as random
295 factors. We used normal distributions with identity links for all models related to acoustic
296 features, except for “syllable repetition rate”, for which we used the Gamma distribution with
297 log link function. All analyses used the *lme4* and *visreg* packages (Bates et al., 2015;
298 Breheny & Burchett, 2017) in R version 3.5 (R Core Team, 2018). Residuals were checked
299 using *DHARMAa* package (Hartig, 2020), multicollinearity and conditional R^2 was tested
300 and estimated with the *performance* package (Lüdecke et al., 2021). A column showing
301 overall sample sizes and sampling effort for each followed group appears in Table 1. For
302 the different response variables, we had different sampling units, as follows: 1) long call
303 occurrence: sampling unit = behavioral bout; 2) number of long calls: sampling unit =
304 behavioral bout with at least one long call; 3) long call acoustic features (i.e. frequency,

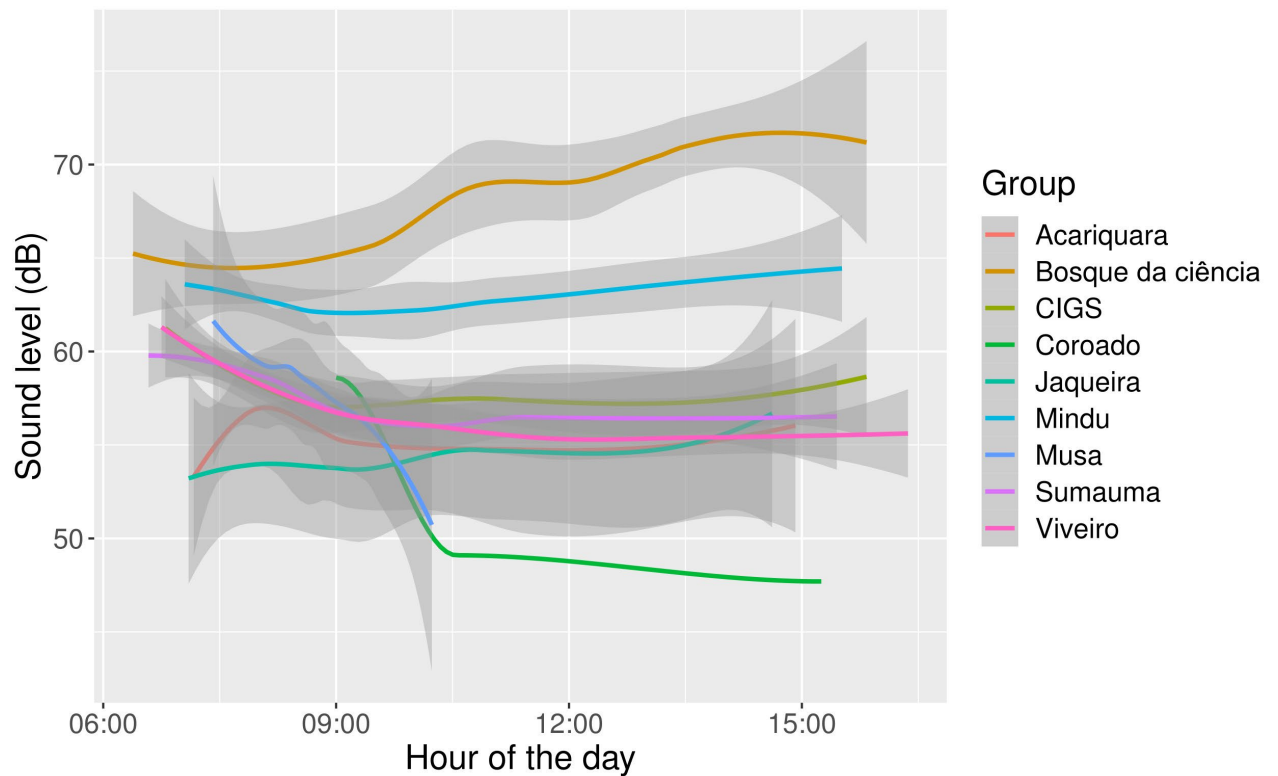
305 duration, syllable repetition rate): sampling unit = recorded long call of sufficient quality to
306 allow analysis.

307 *Ethical approval*

308 The research was approved by the Ethical Committee of the Instituto Nacional de
309 Pesquisas da Amazônia (SEI.01280.00900\2018-58). SISBIO/MMA (Ministry of
310 Environment) granted us the necessary licenses to capture, anesthetize, manipulate, mark,
311 and release the study animals (N. 60347-1). Appropriate permissions were also obtained to
312 access the parks and military areas surveyed. The study was in accordance with ethical
313 standards from the American Society of Primatologists Code of Best Practices for Field
314 Primatology.

315 **Results**

316 The average noise in all sampled areas was 59.19 dB (SD = 5.9) with a standard
317 deviation of 5.54 dB throughout the day (Figure 3), ranging from a minimum of 40.2 dB to a
318 maximum of 84.8 dB. The most common anthropogenic noise source in all areas was road
319 traffic. However, there was also air traffic, park visitors, the talking, screaming, and singing
320 of children and university students, and gunshots from military training activities. Further
321 acoustic sources included biotic sounds such as bird, frogs, and insects. There was some
322 difference in noise distribution across studied groups, although variation across the day was
323 not extensive (Figure 3).



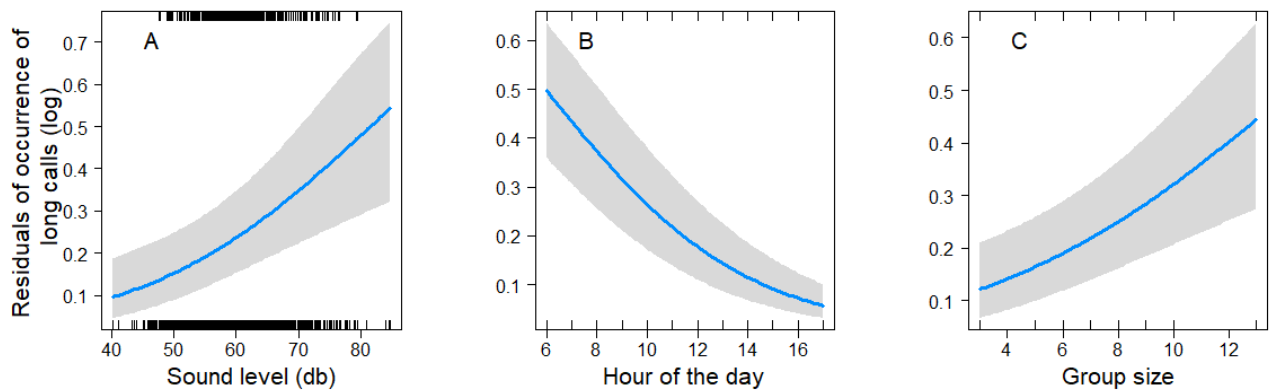
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325 Figure 3 Variation in noise levels throughout the day in areas occupied by different
 326 pied tamarin (*Saguinus bicolor*) groups. Estimates based on 1953 noise estimates of 1 -min
 327 duration.

328

329 *Long-call occurrence (binary data)*

330 We analyzed the probability of animals calling or not. We found that the occurrence
 331 of long calls was positively related to anthropogenic noise and group size, and negatively
 332 related to the time of day (Figure. 4), while the distance from the border of the group home
 333 ranges had no detectable effect on long call occurrence probability (Table 2). The
 334 occurrence of long calls was also related to group size and time of day, but not the
 335 interaction between time of the day and anthropogenic noise (Table 2; Table S1) (Figure .4)
 336 (Table S1).



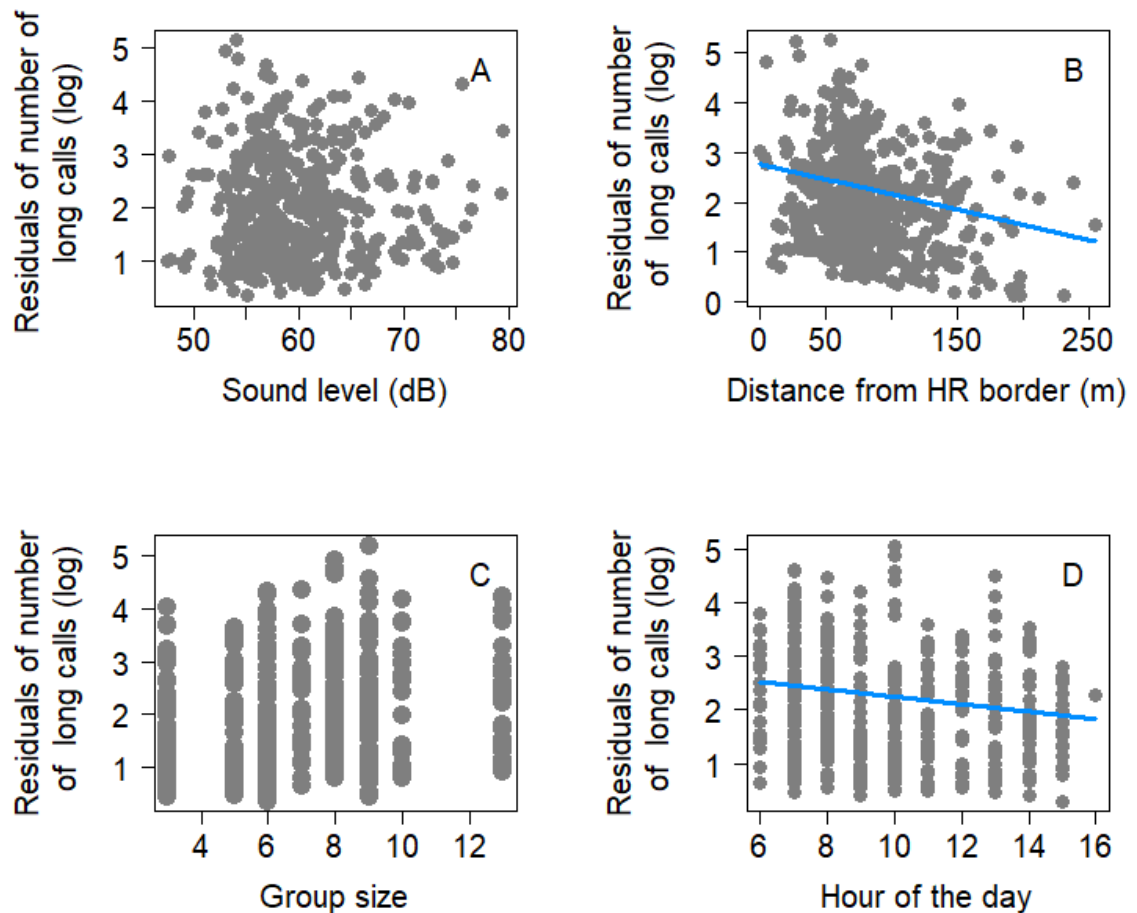
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338 Figure 4 Relation between occurrence of pied tamarin (*Saguinus bicolor*) long calls
 339 (1 = long call occurred; 0 = long call did not occur) and (a) sound levels (dB), (b) hour of
 340 the day, and (c) group size (N = 1953). Lines represent predicted means for each group of
 341 observations from the models presented in Table 2. Plots use partial residuals of the
 342 response variable and show the effect of a given relation while controlling for the effects of
 343 remaining predictors.

344

345 *Number of long calls (count data)*

346 The number of long calls per behavioral bout varied from 1 to 66. Such variation was
 347 not related to anthropogenic noise (Table 2). In contrast, distance from the border of group
 348 home ranges influenced the number of long calls — pied tamarins called more when
 349 distances to neighboring groups were shorter (Figure 5). The number of long calls was also
 350 related to the time of day, with pied tamarins calling more in the early morning, though this
 351 was independent of anthropogenic noise levels or group size (Table 2; Table S1) (Figure 5).



352

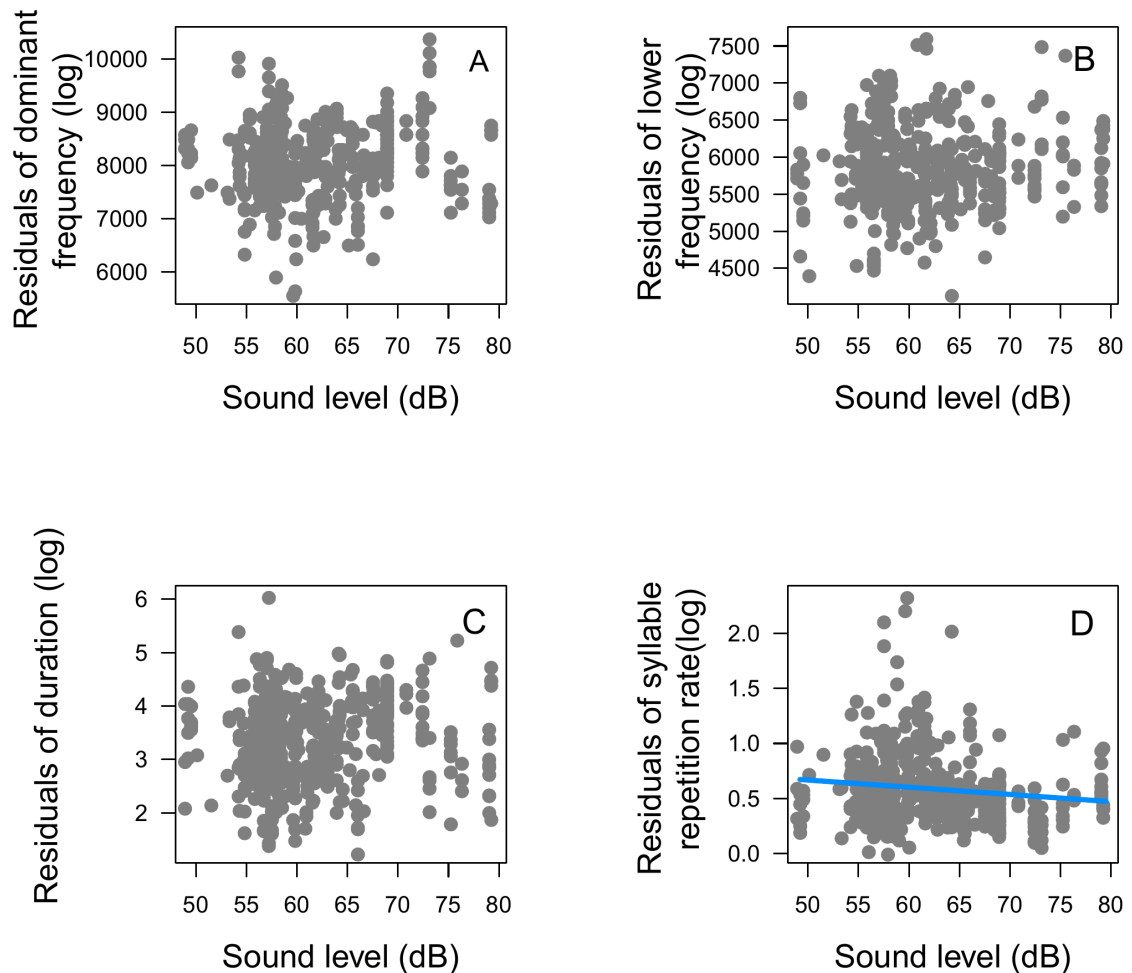
353 Figure 5 Relationships between the number of pied tamarin (*Saguinus bicolor*) long
 354 calls and: (a) sound level; (b) distance from home range border; (c) group size, and (d) hour
 355 of the day. Each point represents a behavioral bout with at least one long call (N = 472).
 356 Lines represent predicted means for each group of observations from the models presented
 357 at Table 2. Plots use partial residuals of the response variable and show the effect of a
 358 given relationship while controlling for the effects of the remaining predictors. The tendency
 359 line is shown only for significant relationships.

360

361 *Long call acoustic features*

362 The lowest and dominant frequency of pied tamarin long calls did not change in
 363 response to anthropogenic noise levels. Similarly, long call duration did not alter in

364 response to anthropogenic sound levels. Syllable repetition rate, on the other hand,
 365 decreased as anthropogenic noise increased (Table 3) (Figure 6).



366

367 Figure 6 Relationship between frequency (a,b) and temporal (c,d) acoustic features
 368 of pied tamarin (*Saguinus bicolor*) long calls and sound level (dB). Each point represents a
 369 long call recording (N = 520). Lines represent predicted means for each group of
 370 observations from the models presented in Table 3. Plots use partial residuals of the
 371 response variable and show the effect of a given relationship while controlling for the effects
 372 of the remaining predictors. The tendency line is shown only for the significant
 373 relationship.

374

375 **Discussion**

376 We found that the occurrence and number of long calls emitted by pied tamarins in
377 urban areas are affected by anthropogenic noise levels, time of day, and distance from
378 neighboring groups. However, pied tamarins did not alter their diel vocal activity across the
379 day in response to anthropogenic noise (Table S1). Patterns of daily vocal activity were
380 independent of sound levels, with individuals calling most often early in the morning. Even if
381 pied tamarins did not change the temporal pattern of daily calling activity in response to
382 anthropogenic noise, fine-scale timing of signalling was influenced by sound level, with pied
383 tamarins calling with a slower syllable repetition rate in noisier areas. Other pied tamarin
384 acoustic features, such as dominant frequency, lowest frequency and long call duration did
385 not change in response to anthropogenic noise.

386 In the current study, we found an average noise of ~59 dB in areas occupied by pied
387 tamarins. For humans, 50-60 dB causes from moderate to serious annoyance (Guski et al.,
388 2017). Unfortunately, we do not have estimates of parameters such as “annoyance” for
389 non-human primates. What is considered “noise” may also depend on a species’ hearing
390 capacities (Clark & Dunn, 2022) and pied tamarin audiograms are currently unavailable.
391 Even though we lack such clearcut values to establish what constitutes noise for pied
392 tamarins, we found that when sound levels were relatively higher, the probability of pied
393 tamarins emitting a long call increased. Since long calls are used for group cohesion and
394 may also help in navigation (Fischer & Zinner, 2011; Snowdon, 2017), it may be that when
395 sound levels surpass a threshold, pied tamarins move to quieter areas. Many studies have
396 shown that loud noises can induce escape responses that influence patterns of both short-
397 and long-range movements, such as the use of space, dispersion, and migration (Duarte et
398 al., 2011; Neo et al., 2014; Tennessen et al., 2014; Velasquez Jimenez et al., 2020; Ware
399 et al., 2015). However, this hypothesis remains to be tested for pied tamarins.

400 Contrary to our predictions, we found no association between the number of pied
401 tamarin long calls and anthropogenic noise levels (Williams et al., 2014). When facing
402 intense noise, pied tamarins do not necessarily call more, likely because this could lead to
403 additional energy expenditure and increase predation risk. Other strategies, such as
404 changes in syllable repetition rate (see below), and complimenting their communication with
405 other communication channels, including olfaction (Sobroza et al. 2023), could be sufficient
406 to circumvent the deleterious effects of increased noise (Bejder et al., 2009). In noisier
407 places, the pied tamarins' long calls had reduced syllable repetition rates, meaning that
408 they emitted slower long calls, with either time between syllables being longer or the
409 syllables themselves being more prolonged. A longer inter-syllable duration could allow
410 pied tamarins to call during short periods when noise levels were reduced, a feature also
411 observed with captive cotton-top tamarins (*Saguinus oedipus*) and common marmosets
412 (*Callithrix jacchus*) that called between bursts of loud noises (Egnor et al., 2007; Roy et al.,
413 2011). If in pied tamarins, the syllables function as a unit of information, as in cotton-top
414 tamarins (Miller et al., 2003), and the lower syllable repetition rate implies that the syllables
415 are longer, this could also be advantageous as longer signals would have more chance to
416 reach the receiver.

417 As predicted, we did not find a difference in frequency features of long calls in
418 response to sound levels, possibly because pied tamarins sounds are already high-pitched
419 (Sobroza et al., 2017). This is different from the situation described for other callitrichids
420 which modulate frequency features rapidly (Zhao et al., 2019). Analysis of the frequency
421 contours of each syllable in relation to the spectral composition of background noise could
422 be instructive in this context. Also, many species increase the amplitude of their calls
423 (Lombard effect) to boost the signal-to-noise ratio and enhance signal transmission
424 capacity (Brumm & Zollinger, 2011). We did not analyze amplitude because it requires

425 different field techniques (Zollinger et al., 2012). However, this is a strategy widely used by
426 wildlife in response to noise (Brumm & Zollinger, 2011) and pied tamarins could well be
427 using this too.

428 Our data also did not corroborate the hypothesis that pied tamarins alter their daily
429 vocal activity in response to anthropogenic noise. However, individuals of this species
430 vocalize more during the morning, which is also a widespread pattern among birds and
431 primate species (Schneider et al., 2008; Van Belle et al., 2013), supposedly because
432 temperature, humidity and wind conditions are generally more favorable to sound
433 propagation at this time (Waser & Brown, 1984). However, these propagation capacities are
434 usually tested in pristine areas, and the distinct microclimate and the ubiquity of planar
435 surfaces in urban areas could cause subtle changes to acoustic propagation (Slabbekoorn
436 et al., 2007). An alternative hypothesis for the peaking of vocal activity during the morning
437 relies on pied tamarin diel behavior: individuals usually enter their sleeping sites relatively
438 early in the afternoon (~15:30 -16:00) and begin their daily activities around 05:30 (Egler,
439 1986; Gordo et al., 2017). After more than 12 hours of resting, individuals must be hungry,
440 and long calls could be important to coordinate the group when searching for fruits.

441 We also found that social aspects are important predictors for the occurrence and
442 number of long calls. As expected, we found that the occurrence of long calls was related to
443 group size, and there was no evidence of a correlation between group size and the number
444 of long calls. Such a finding is in accordance with a previous pied tamarin playback study
445 which showed that, unlike other group-living animals (Kitchen, 2004; Van Belle & Scarry,
446 2015), larger groups of pied tamarins do not emit more long calls when exposed to
447 conspecific long calls (Sobroza, Gordo, Barnett, et al., 2021). We recognize that our
448 sampling size is relatively small as each group had a different number of individuals (Table
449 1). However, in our study, we included group identity as a random factor in the model to

450 account for the non-independence of observations. We also found a negative relationship
451 between the number of long calls and the distance from home range boundaries. This result
452 matches findings from other territorial species that patrol their territories regularly; signalling
453 occurs more at territorial boundaries (Da Cunha & Jalles-Filho, 2007; Mitani & Watts, 2005).
454 The models we proposed to evaluate effect of different predictors on the occurrence and
455 number of long calls lacked explanatory power, accounting for only 18% to 21% of the data
456 (Table 2). It is possible that other variables not included in the current study, such as
457 distance between group members and resource availability, may have an important role
458 and should be considered in future studies.

459 It is important to note that in our study, we characterized noise in terms of amplitude.
460 However, noise is complex and variable in terms of spectral and temporal signatures (Clark
461 & Dunn, 2022; Gill et al., 2015; Neo et al., 2014). Howler monkeys, for instance, respond
462 more to aerial traffic than other human-produced noises (Gómez-Espinosa et al., 2022).
463 This trend is possibly related to the nature of their low-pitched calls that coincide with the
464 frequencies in which aerial traffic has the most energy. Perhaps, as high-pitched callers,
465 pied tamarins would present a different pattern of response towards different noise
466 categories with varying frequency profiles (Gómez-Espinosa et al., 2022). We did not
467 directly evaluate the composition of the soundscape, thus our “noise” estimates include not
468 only anthropophonies (i.e. sounds of anthropogenic origin), but also biophonies (i.e. sounds
469 of biological origins, such as other animals calling or moving). For instance, cicadas are an
470 important component in tropical soundscapes as their sounds can achieve very loud
471 amplitudes, often occupy a large frequency band, and they can produce sounds for several
472 minutes and even hours, affecting the overall use of the acoustic space (Aide et al., 2017).
473 Future studies should consider the composition of noise, to disentangle the different
474 impacts of amplitude vs. spectral and temporal features.

475 In summary, pied tamarins do not increase the long call rate in response to noise,
476 but the occurrence of long calls is influenced by sound levels. As long calls are important
477 for group cohesion during movement, it is possible that the tamarins emit a single call with a
478 changed acoustic structure (i.e. lower syllable repetition rate and probably higher
479 amplitude). Neither occurrence nor the number of long calls were altered to occur in quieter
480 periods of the day, although, independent of sound levels, the emission of long calls is
481 more likely to occur early in the morning. Further, the number of long calls was negatively
482 related to the distance to home range boundaries. Overall, pied tamarins are likely to adjust
483 their vocal behavior and call features in a way that could possibly allow for effective
484 communication in urban settings. Which, from a long-term perspective, could enable this
485 Critically Endangered species to continue surviving in the largest city in the Brazilian
486 Amazon.

487 **Author contributions**

488 T.V. Sobroza, A.A. Barnett, M. Gordo and J.C. Dunn originally formulated the
489 research topic. T.V. Sobroza raised funds, collected data, and wrote the original draft. B.M.
490 Naissinger and T.V. Sobroza performed exploratory and current spatial analysis, P.
491 Pequeno and T.V. Sobroza performed statistical analyses, A.A. Barnett, M. Gordo, and J.C.
492 Dunn supervised. A.A. Barnett and J.C. Dunn checked the English. All authors contributed
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503 **Data availability statement**

504 The data that support the findings of this study are available from the corresponding
 505 author upon request.

506 **Conflict of interest**

507 The authors declare no conflict of interest.

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853 Table 1. Characteristics of sampling locations, sampling sizes and pied tamarin (*Saguinus*
 854 *bicolor*) groups followed in urban areas of Manaus, Amazonas State, Brazil.

Group	Approximate Fragment size (ha)*	Group Size	Group composition	Number of behavioral bouts recorded	Number of long call recordings
Viveiro	700	8	1F + 2M + 3JF + I + 1UN	403	20
Jaqueira	700	13	3F + 5M + 2JF + 1JM + 1I + 1UN	60	30
Coroado	700	7	3M + 1JM + 1I + 2UN	54	0
Acariquara	700	7	2F + 1 JF + 1 MF + 3UN	43	47
CIGS	100	5	1F + 2M + 1FJ + 1 UN	440	58
Mindu	50	6	2F + 1M + 1MJ + 2 UN	215	61
Sumaúma	50	9	3F + 3M + 1FJ + 2MJ	418	106
Bosque da Ciência	24	3	2F + 1M	275	117
Musa	continuous	10	UN	45	5

855 * Fragment size estimate is not precise as some of the fragments are more or less
 856 connected with other forested areas by gardens or riverside vegetation. Group composition:
 857 M= adult male; F= adult female; JF= juvenile female; JM= juvenile male; I=infant;
 858 UN=adults of unknown sex.

859

860

861 **Table 2.** Summary of Generalized Mixed Effects Models for different descriptors of
 862 emissions of long calls by nine pied tamarin (*Saguinus bicolor*) groups in urban areas of
 863 Manaus, Brazil.

Response variable	Predictor	Estimate	SE	Z	p
Occurrence of long calls <i>(binary data)</i>	Intercept	-2.997	1.088	-2.755	<0.001
	Sound level (dB)	0.054	0.014	3.623	<0.001
	Hour of the day	-0.253	0.021	-11.57	<0.001
	Group size	0.175	0.0452	3.879	<0.001
	Distance from HR border	0.001	0.001	1.068	0.285
	<i>Marginal R² / Conditional R²</i>	0.167/ 0.182			
Number of long calls <i>(count data)</i>	Intercept	3.036	0.916	3.313	<0.001
	Sound level (dB)	-0.003	0.012	-0.289	0.772
	Hour of the day	-0.069	0.018	-3.843	<0.001
	Group size	0.070	0.042	1.683	0.092
	Distance from HR border	-0.006	0.001	-4.202	<0.001
	<i>Marginal R² / Conditional R²</i>	0.12/ 0.21			

864 HR= home range.

865

866 **Table S1.** Summary of Generalized Mixed Effects Models including interaction factors for
 867 different descriptors of emissions of long calls by nine groups of pied tamarin (*Saguinus*
 868 *bicolor*) in urban areas of Manaus, Brazil.

Response variable	Predictor	Estimate	SE	Z	p
Occurrence of long calls <i>(binary data)</i>	Intercept	-0.655	2.893	-0.22	0.820
	Sound level (dB)	0.015	0.047	0.32	0.748
	Hour of the day	-0.466	0.244	-1.92	0.055
	Group size	0.172	0.045	3.79	<0.001
	Distance from HR border	0.0018	0.001	1.11	0.262
	Sound level (dB) × Hour of the day	0.003	0.004	0.878	0.380
Number of long calls rate <i>(count data)</i>	Intercept	4.336	2.131	2.03	0.041
	Sound level (dB)	-0.022	0.035	-0.64	0.516
	Hour of the day	-0.176	.0184	-0.96	0.337
	Group size	0.04	0.033	1.22	0.22
	Distance from HR border	-0.004	0.001	-3.29	<0.001
	Sound level (dB) × Hour of the day	0.001	0.003	0.52	0.59

869 HR = home range; × Interaction term

870

871

872 **Table 3.** Summary of Generalized Mixed Effects Models for different descriptors of
 873 emissions of long calls by nine pied tamarin (*Saguinus bicolor*) groups in urban areas of
 874 Manaus, Brazil.

Response variable	Predictor	Estimate	SE	Z	p ⁸⁷⁵
Dominant frequency	Intercept	7548.51	561.38	13.44	<0.01
	Sound level (dB)	7.789	8.32	0.93	0.35
Lowest frequency	Intercept	5713.75	421.32	13.56	<0.01
	Sound level (dB)	3.15 6	6.434	0.49	0.624
Duration	Intercept	2.389	0.557	4.283	<0.01
	Sound level (dB)	0.111	0.099	1.239	0.215
Syllable repetition rate	Intercept	0.925	0.133	6.955	<0.001
	Sound level (dB)	-0.006	0.002	-3.227	<0.01*

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877