



**HAL**  
open science

## Honest signaling in mouse lemur vocalizations?

Claudia Fichtel, Peter M Kappeler, Martine Perret, Elise Huchard,  
Pierre-Yves Henry

► **To cite this version:**

Claudia Fichtel, Peter M Kappeler, Martine Perret, Elise Huchard, Pierre-Yves Henry. Honest signaling in mouse lemur vocalizations?. *International Journal of Primatology*, 2022, 43, pp.752-773. 10.1007/s10764-021-00265-9 . hal-03463866

**HAL Id: hal-03463866**

**<https://hal.umontpellier.fr/hal-03463866>**

Submitted on 2 Dec 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title: Honest signaling in mouse lemur vocalizations?**

2

3 Claudia Fichtel<sup>1,2</sup>, Peter M. Kappeler<sup>1,3</sup>, Martine Perret<sup>4</sup>, Elise Huchard<sup>5</sup>, Pierre-Yves Henry<sup>4</sup>

4

5 <sup>1</sup>Behavioral Ecology & Sociobiology Unit, German Primate Center, Leibniz Institute for Primate  
6 Research Kellnerweg 4, 37077 Göttingen, Germany

7 <sup>2</sup>Leibniz-ScienceCampus Primate Cognition, Göttingen, Germany

8 <sup>3</sup>Department of Sociobiology/Anthropology, Johann-Friedrich-Blumenbach Institute of  
9 Zoology and Anthropology, Georg-August Universität, Göttingen, Germany

10 <sup>4</sup>Mécanismes Adaptatifs et Evolution (MECADEV - UMR 7179), CNRS, MNHN, Brunoy, France

11 <sup>5</sup>Institute of Evolutionary Biology of Montpellier (ISEM), Université de Montpellier, CNRS,  
12 Montpellier, France

13

14

15 **Corresponding author:** Email: [Claudia.fichtel@gwdg.de](mailto:Claudia.fichtel@gwdg.de)

16

17

18

19 **Abstract**

20 Animal vocalizations may provide information about a sender's condition or motivational  
21 state and, hence, mediate social interactions. In this study, we examined whether  
22 vocalizations of gray mouse lemurs (*Microcebus murinus*) emitted in aggressive contexts  
23 (grunts, tsaks) co-vary with physical condition, which would underly and indicate honest  
24 signaling. We recorded calls from captive individuals that were subjected to a caloric restricted  
25 (CR) or *ad libitum* (AL) diet, assuming that individuals on an *ad libitum* dietary regime were in  
26 better condition. We analyzed 828 grunts produced by 7 CR and 9 AL individuals and 270 tsaks  
27 by 8 CR and 5 AL individuals. Grunts consisted of two separate elements, with the 1<sup>st</sup> element  
28 having more energy in higher frequencies than the 2<sup>nd</sup> element. Body mass correlated  
29 negatively with acoustic features of grunts, and heavier individuals produced lower-frequency  
30 grunts. Acoustic features of grunts did not differ between sexes. Acoustic features of tsaks  
31 were predicted by neither body mass nor sex. However, tsaks produced by AL individuals were  
32 noisier than those of CR individuals. Hence, manipulation of body condition via dietary  
33 regimes affected acoustic features of calls given during aggression in different ways: acoustic  
34 features of grunts varied according to the rule of acoustic allometry, and can be considered as  
35 honest signals. Acoustic features of tsaks, however, varied according to motivational  
36 structural rules. Longitudinal studies are now indicated to examine whether intra-individual  
37 changes in body mass are also reflected in the acoustic structure of calls, allowing callers to  
38 signal more flexible variation in condition.

39

40 **Keywords:** communication, honest signaling, acoustic allometry, motivational structural  
41 rules, vocalizations, primates, mouse lemurs

42

43

44

## 45 **Introduction**

46 A central objective in animal communication research is to understand what information is  
47 conveyed in signals, and what function communication may serve during social interactions  
48 (Cheney and Seyfarth 2003). Among a wide range of taxa, acoustic cues in vocal signals are  
49 associated with rather stable, long-term attributes of callers, such as individual or species  
50 identity, sex, age, kin, and body size (Charlton *et al.* 2020; Clarke *et al.* 2006; Ey *et al.* 2007;  
51 Fichtel 2014; Masters *et al.* 1995; Rendall *et al.* 1996; Ryan and Brenowitz 1985; Zimmermann  
52 *et al.* 2000). In contrast, acoustic cues in vocalizations are also associated with more flexible  
53 attributes of the caller, such as their motivational (Fichtel *et al.* 2001; Fichtel and  
54 Hammerschmidt 2002; 2003; Scheumann *et al.* 2007) or physiological state (Barelli *et al.* 2013;  
55 Buesching *et al.* 1998; Charlton *et al.* 2010a; Semple *et al.* 2002; Zimmermann 1996),  
56 dominance status (Kitchen *et al.* 2003; Vannoni and McElligott 2008), or fighting ability  
57 (Fischer *et al.* 2004; Reby and McComb 2003; Reby *et al.* 2005).

58 Fighting ability is often operationalized through body size, and it is well established that  
59 frequencies in vocalizations co-vary with body size across frogs, birds and mammals (Bowling  
60 *et al.* 2017; Charlton and Reby 2016; Fitch and Hauser 1995; Garcia *et al.* 2018; Gingras *et al.*  
61 2013; Hauser 1993; Wallschläger 1980). According to this rule of acoustic allometry, larger  
62 animals tend to produce lower-frequency calls than smaller animals, because they have longer  
63 vocal tracts producing lower resonances and longer vocal folds oscillating at lower frequencies  
64 (Fitch 1997; Taylor and Reby 2010). For example, among primates and carnivores, the  
65 fundamental frequency and the frequency with the maximum amplitude correlate negatively  
66 with body size (Bowling *et al.* 2017). Among primates, the fundamental frequency correlates  
67 negatively with body size and vocal fold length, but the latter predicts more precisely the  
68 fundamental frequency, indicating a potential for decoupling between larynx and body size  
69 (Garcia *et al.* 2018). Such a decoupling of larynx and body size has been documented in several  
70 mammalian species (Charlton and Reby 2016). For example, the hypertrophied larynges in  
71 howler monkeys (*Alouatta* spp.) allow them to produce much lower formant frequencies than  
72 expected for their body size (Dunn *et al.* 2015).

73 Acoustic cues that provide accurate information about animal attributes, such as body  
74 size, may convey honest information because only individuals in better condition are capable  
75 to bear any costs associated with signal production (Zahavi 1977). Accordingly, vocal  
76 performance has been suggested to reflect individual quality (Bradbury and Vehrencamp

77 2011). Since body mass reflects individual's fighting ability (Arnott and Elwood 2009; Jonart *et*  
78 *al.* 2007; Vieira and Peixoto 2013), acoustic correlates of these traits are biologically relevant  
79 for senders and receivers. For example, giant pandas (*Ailuropoda melanoleuca*) produce  
80 'bleats' when they encounter and compete over estrous females that encode cues about the  
81 caller's sex, age and body size (Charlton *et al.* 2009). Playback experiments with bleats showed  
82 that males as well as females respond more strongly to calls indicating larger male size  
83 (Charlton *et al.* 2010b). Similarly, male red deer (*Cervus elaphus*) respond more strongly to  
84 acoustic features of roars that indicate larger size and better fighting ability (Reby *et al.* 2005).  
85 Finally, among anthropoid primates, sexual dimorphism in fundamental frequency is  
86 associated with variation in the mating system. It increases during evolutionary transitions  
87 towards polygyny and decreases with transitions towards monogamy, suggesting that low  
88 male fundamental frequencies signal condition and have evolved in response or in parallel to  
89 changes in the intensity of mating competition (Puts *et al.* 2016).

90 Many species experience periodic changes in body mass due to variable food availability  
91 in the lean versus the rich season, but little is known about how such changes are reflected in  
92 the acoustic structure of animal vocalizations and whether these dynamics represent flexible  
93 indicators of fighting ability. Mouse lemurs are a suitable study species for this question  
94 because they have to cope with low food availability and high climatic unpredictability in their  
95 natural environment in Madagascar (Dewar and Richard 2007). During the dry season, the  
96 austral winter, mouse lemurs experience shortfalls in food availability and lose body mass  
97 accordingly (Dammhahn and Kappeler 2008; Schmid and Kappeler 2008). However, seasonal  
98 body and tail fattening in combination with energy saving strategies such as torpor, which is  
99 photoperiodically controlled, enables them to face these unfavourable environmental  
100 conditions (Aujard *et al.* 1998; Schmid and Kappeler 1998; Vuarin *et al.* 2013). As a  
101 consequence, mouse lemurs experience fluctuations of about one third in body mass across  
102 winter and summer in both captivity and the wild (Perret and Aujard 2001; Schmid and  
103 Kappeler 1998).

104 Moreover, mouse lemurs are sexually monomorphic, nocturnal, solitary foragers  
105 exhibiting an unusual mating system; they are polygnyandrous and females are sexually  
106 receptive for only a few hours once a year (Eberle and Kappeler 2004a; Schmid and Kappeler  
107 1998). In the wild, body mass crucially impacts mating strategies of males, with heavier males  
108 having higher reproductive success (Eberle and Kappeler 2004b). In captivity, copulation

109 success, however, is not influenced by body mass but by male competitiveness and aggression  
110 (Gomez *et al.* 2012). In addition, females in better condition are more polyandrous than  
111 females in weaker condition, which might result in elevated genetic diversity or quality of  
112 offspring (Huchard *et al.* 2012; Jennions and Petrie 2000). Hence, signalling condition in  
113 acoustic displays during competition over and with mates might be beneficial for both sexes.  
114 Males may benefit by signalling condition to deter rival males but also to advertise quality to  
115 females, which preferentially mate with more competitive and aggressive males (Gomez *et al.*  
116 2012). Since up to 14 males can solicit matings with an oestrous female, females may also  
117 benefit by signalling condition to deter unwanted mating attempts of males (Eberle and  
118 Kappeler 2004 a,b; Huchard *et al.* 2012).

119 During agonistic interactions, mouse lemurs produce grunts and tsaks and sometimes  
120 whistles. Grunts are considered as defensive threat calls, given during disturbances at their  
121 sleeping sites and during aggressive interactions (Leliveld *et al.* 2011; Zimmermann 2010).  
122 Tsaks are given during aggressive interactions with conspecifics but also during encounters  
123 with predators (Rahlfs and Fichtel 2010; Zimmermann 2010). Whistles are harmonic calls that  
124 are given in several contexts and may serve to recruit conspecifics (Radespiel 2000; Rahlfs and  
125 Fichtel 2010; Zimmermann 1996).

126 During the mating season mouse lemurs produce so-called trills that serve to attract  
127 mates. In males, calling rate of trills is associated with rank and may serve to advertise quality  
128 (Zimmerman 1996; Buesching *et al.* 1998). Hence, it is likely that also call types given during  
129 agonistic interactions may contain cues about the sender's quality. We, therefore,  
130 investigated whether caloric reduction influences acoustic features of vocalizations in gray  
131 mouse lemurs (*Microcebus murinus*). We recorded vocalizations given during agonistic  
132 contexts in captive mouse lemurs that were exposed to different dietary regimes, i.e. an *ad*  
133 *libitum* and caloric restricted diet. These dietary restriction experiments were implemented  
134 to study the effect of caloric restriction on physiology, cognitive abilities or female mating  
135 strategies (Canale *et al.* 2011; Giroud *et al.* 2008; Huchard *et al.* 2012). According to the rule  
136 of acoustic allometry, we predicted that mouse lemurs that were exposed to a dietary  
137 restriction, and, hence, assumed to be in a weaker condition, should produce higher-  
138 frequency calls than those exposed to an *ad libitum* diet. Since mouse lemurs are  
139 monomorphic, we did not predict that acoustic features co-vary with sex.

140

141 **Methods**

142 *Subjects*

143 Subjects were housed under standard breeding conditions in the breeding colony at the  
144 Muséum National d'Histoire Naturelle in Brunoy, France, IBISA platform, agreement  
145 E91.114.1, DDPP Essonne. Animals were kept with 14h of light and 10h of dark during the six  
146 month period of long days and 10h of light and 14h of dark during the six month period of  
147 short days (Perret and Aujard 2001). Animals were kept under two different feeding regimes:  
148 *Ad libitum* (AL), where animals were fed with a homemade standard diet with a caloric value  
149 of  $4.8 \text{ kJg}^{-1}$ , containing 50% carbohydrates, 20% proteins, and 30% lipids. In the caloric  
150 restricted (CR) regime, animals were exposed to a 80% caloric restriction with a daily food  
151 supply of 20% of the food mass offered to the group under the *ad libitum* regime (Giroud *et al.*  
152 *2008*; Huchard *et al.* 2012). CR individuals were housed individually in cages (50 cm x 50  
153 cm x 50 cm) with branches and two nest-boxes to control caloric intake. Studies of the short-  
154 and long-term effects of caloric restriction on the endocrine system and energy balance  
155 revealed that mouse lemurs have pronounced physiological flexibility, enabling them to adapt  
156 to acute food shortage (Giroud *et al.* 2008; Canale *et al.* 2011; Dal-Pan *et al.* 2011). Notably,  
157 the food-restricted individuals were not found to experience chronic stress, as urinary cortisol  
158 excretion (Canale *et al.* 2011) and plasma testosterone levels (Dal-Pan *et al.* 2011) remained  
159 unaffected by dietary treatment.

160 We recorded and analyzed the vocalizations of 25 individuals (13 AL and 12 CR, Table 1)  
161 during mild disturbances at the sleeping box, i.e., when animals were taken out of their home  
162 cage in their sleeping box to either clean the cage or to inspect their well-being. During this  
163 procedure, we opened the door of the sleeping box to about one third of the full opening and  
164 positioned the microphone about 30 cm in front of it.

165 Some individuals were related with a maternal relatedness coefficient ranging from  
166 0.125-0.5. For grunts, 2 of 36 dyads were related in the AL condition, whereas none of the  
167 dyads (N=28) was related in the CR condition. For tsaks, 2 of 10 dyads were related in the AL  
168 condition, whereas only 3 of 36 dyads were related in the CR condition. Since only a few dyads  
169 were related, we did not control for relatedness in the statistical analyses.

170

171

172

173 Table 1: Individual identity, sex, diet and number of calls analyzed in a study of mouse lemur  
 174 vocalizations housed at the CNRS, MNHN, Brunoy (2010).

Diet	Sex	ID	N Grunts		N Tsaks
			1 <sup>st</sup> element	2 <sup>nd</sup> element	
AL	F	1	27	26	-
AL	F	4	30	30	-
AL	F	5	-	-	6
AL	F	6	-	-	19
AL	F	7	28	18	18
AL	F	8	20	17	-
AL	F	12	20	11	-
AL	F	13	29	16	-
AL	M	16	-	-	30
AL	M	18	-	-	21
AL	M	20	27	22	-
AL	M	21	22	12	-
AL	M	22	27	7	-
CR	F	2	53	51	14
CR	F	3	23	23	-
CR	F	9	41	35	15
CR	F	10	23	21	31
CR	F	11	54	45	-
CR	M	14	-	-	30
CR	M	15	-	-	24
CR	M	17	-	-	24
CR	M	19	-	-	25
CR	M	23	20	15	-
CR	M	24	23	12	-
CR	M	25	-	-	13

175  
 176  
 177  
 178  
 179  
 180  
 181  
 182  
 183  
 184



185 **Recordings and acoustic analyses**

186 We recorded vocalizations with an ultrasound microphone (polarized condenser microphone  
187 CMPA, Avisoft, Germany) in combination with an Avisoft-UltraSoundGate 116 (Avisoft,  
188 Germany) recording device resulting in a frequency range of 20-200 kHz that was connected  
189 to a Toshiba Satellite Pro laptop (Toshiba Europe GmbH, Germany) with 'AVISOFT-RECORDER  
190 v.2.96' software (Avisoft, Germany). We digitized call recordings with a sampling rate of 500  
191 kHz at 16 bit resolution and converted them into spectrograms with 1024-point fast Fourier  
192 transforms (window function: Hanning, 93.75% overlap; time resolution: 0.64 ms; frequency  
193 range: 100 kHz) using AVISOFT-SASLab Pro 4.2 (Avisoft, Germany). We visually inspected and  
194 sampled only calls of good quality and low background noise for acoustic analysis. We derived  
195 acoustic variables from frequency-time spectra with a time resolution of 5 ms for each time  
196 window across the call using LMA 9.2, a custom software tool to extract different sets of  
197 variables from acoustic signals (Schrader and Hammerschmidt 1997).

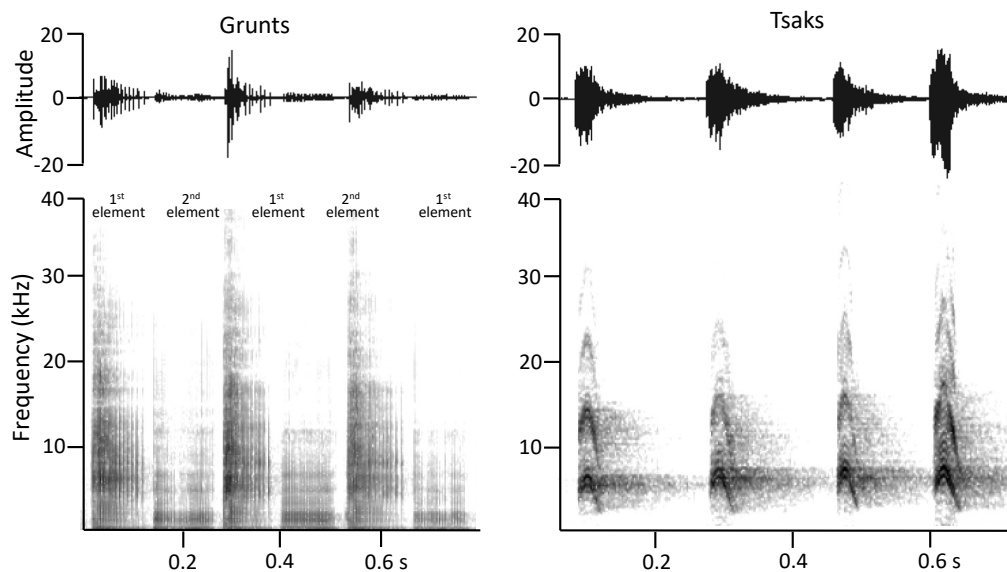
198 To avoid potential effects of a group-signature in the acoustic structure of vocalizations,  
199 we recorded vocalizations from AL individuals that were housed in different groups and kept  
200 CR individuals in individual cages in different rooms. Mouse lemurs produced three different  
201 call types during these mild disturbances: grunts, tsaks, and whistles. Grunts are noisy low-  
202 frequency calls, that consist of two clearly separated elements which are usually uttered  
203 together, but sometimes mouse lemurs produce only the first element (Fig. 1a). Therefore,  
204 we digitized and analyzed the two elements separately, referring to them as the 1<sup>st</sup> and 2<sup>nd</sup>  
205 elements of grunts. Tsaks are harmonic calls which are commonly described as chevron-  
206 shaped, because they increase in frequency, up to a local peak, followed by a decrease in  
207 frequency (Fig. 1b). Whistles are harmonic calls that are given in several contexts and may  
208 serve to recruit conspecifics in the mating season, when meeting members of sleeping  
209 associations at the sleeping site (Radespiel 2000), or during an escalating predator encounter  
210 to recruit other individuals to mob the predator (Rahlfs and Fichtel 2010). Since only a few  
211 individuals produced whistles (N=6), we did not include them in the analysis.

212 To characterize the acoustic structure of grunts and tsaks (Fig. 1a, b), we measured  
213 acoustic variables that characterize the general call structure and are comparable with  
214 acoustic variables measured in other studies characterizing the structure of mammalian  
215 vocalizations (Fichtel and Hammerschmidt 2002; Fichtel *et al.* 2005; Manser *et al.* 2001). Since  
216 the fundamental frequency can only be measured for vocalizations that are produced by

217 regular vocal fold vibration, we could not measure it in grunts. Because tsaks are noisy calls  
218 that also contain harmonic energy, we could only measure the fundamental frequency reliably  
219 in 83 of 270 calls. Instead, we measured the frequency with the maximum amplitude (peak  
220 frequency) for both calls, which has been suggested to be associated with body size in several  
221 primates and carnivores (Bowling *et al.* 2017).

222

223



224

225 Figure 1: Spectrograms and oscillograms of grunts and tsaks of mouse lemurs housed at the  
226 CNRS, MNHN, Brunoy (2010). For grunts, two examples of calls with the 1<sup>st</sup> and 2<sup>nd</sup> element  
227 and one example with only the 1<sup>st</sup> element are shown.

228

229 For each element of grunts, we measured the duration, the mean frequency range, the mean  
230 peak frequency, the mean lower, central, and upper frequency distribution, as well as the 2<sup>nd</sup>  
231 dominant frequency band (Table 2). To characterize the acoustic structure of tsaks, we also  
232 measured the duration, the mean frequency range and the mean peak frequency. Because  
233 tsaks have a narrow frequency bandwidth, we measured only the central frequency but  
234 included measurements of the start, end and maximum of the central frequency to  
235 characterize the chevron-shaped acoustic structure of these calls. Because tsaks are noisy calls  
236 containing harmonic elements, we also measured the percentage of noisy parts in the call  
237 (Table 2).

238

239

240 Table 2: Definitions of acoustic variables measured for grunts and/or tsaks produced by mouse  
 241 lemurs housed at the CNRS, MNHN, Brunoy (2010).

Acoustic variable	Definition	Call type
Duration (ms)	Time between the onset and end of call	Grunts, tsaks
Mean frequency range (kHz)	Difference between the maximum and minimum frequency	Grunts, tsaks
Mean lower frequency (kHz)	Distribution of frequency amplitudes across the spectrum obtained by calculating the cumulative sum of the frequency amplitudes per time segments. Frequency at which the distribution reached the 1. quartile (25%), averaged across time segments.	Grunts
Mean central frequency (kHz)	Frequency at which the distribution of frequency amplitudes across the spectrum reached the 2. quartile (50%), mean value over all time segments	Grunts, tsaks
Start, end, maximum central frequency (kHz)	Frequency of the central frequency at the beginning and the end of the call as well as the maximum, the highest frequency of central frequency	Tsaks
Mean upper frequency (kHz)	Frequency at which the distribution reached frequency amplitudes across the spectrum reached the 3. quartile (75%), mean value over all time segments	Grunts
2 <sup>nd</sup> dominant frequency band (kHz)	The dominant frequency bands are characterized by amplitudes that exceed a given threshold in a consecutive number of frequency bins.	Grunts
Mean peak frequency (kHz)	Mean frequency with the highest amplitude across time segments	Grunts, tsaks
% noise	Percentage of time segments in which no harmonic structure could be detected	Tsaks
Mean fundamental frequency (kHz)	Mean lowest frequency across tonal time segments	Tsaks

242

### 243 **Statistical analyses**

244 Because some individuals produced only grunts or tsaks and some produced both call types,  
 245 we used a Fisher exact test to investigate whether the number of individuals producing either  
 246 only one call type or both varies as a function of the dietary regime. We tested for differences

247 in body mass of individuals exposed to the two dietary regimes with an exact Mann Whitney  
248 U test.

249 To summarize multiple acoustic variables of a call into a single composite index, we first  
250 ran a Principal Component Analysis (PCA) using the package “rela” (Chajewski 2009). We  
251 included only acoustic variables that describe the distribution of frequencies and not the  
252 duration of calls and log-transformed values of the acoustic variables. The PCAs were justified  
253 as shown by the Kaiser-Meyer-Olkin measure of sampling adequacy (grunts: KMO=0.82; tsaks:  
254 KMO=0.80) and Bartlett's test of sphericity (grunts:  $p < 0.01$ ; tsaks:  $p < 0.001$ ).

255 To estimate the influence of body mass, sex and call element (1<sup>st</sup> and 2<sup>nd</sup> element) on  
256 variation in the acoustic structure of grunts, we fitted two linear mixed models (LMMs) with  
257 either the first (PC1) or second (PC2) extracted principal component as the response variable  
258 using the packages “lme4” and “lmerTest” (Bates *et al.* 2015; Kuznetsova *et al.* 2017). We  
259 fitted body mass, sex, and call element as fixed factors, individual identity as a random  
260 intercept effect, and a random slope of call element (Schielzeth and Forstmeier 2009; Barr *et*  
261 *al.* 2013).

262 To estimate the influence of body mass and sex on variation in the acoustic structure of  
263 tsaks, we fitted another two LMMs with PC1 or PC2 as the response variable, body mass and  
264 sex as fixed factors and individual identity as random factor. For calls in which we could  
265 measure the fundamental frequency, we calculated the mean and correlated it with body  
266 mass using a Spearman rank correlation. Since we could not include body mass and diet in the  
267 models because they were collinear, we fitted four additional models for grunts and tsaks  
268 including diet instead of body mass. We estimated the effect of diet *per se* because in another  
269 study, caloric restricted females mated only with one male, whereas *ad libitum* fed females  
270 mated with several males (Huchard *et al.* 2012). Since mating is costly and body mass loss  
271 during mating correlated with the number of mating partners (Huchard *et al.* 2012), caloric  
272 restricted females might be less likely to engage in energetic costly behavior, such as  
273 displaying aggression during disturbances. In these models we set either PC1 or PC2 as  
274 response variable, diet and sex as fixed factors, individual identity as random factor, and call  
275 element as random slope for models on acoustic variation of grunts. Following Perneger  
276 (1998), we did not apply corrections for multiple testing.

277 We conducted all statistical tests in R (version 4.0.3; R Core Team 2020). For all models  
278 (LMM), we checked the assumptions of normality distributions and homogeneity by visual

279 inspection of a QQ-plot of residuals and residuals plotted against fitted values (Queen *et al.*  
280 2002). We assessed model stability through the level of estimated coefficients and standard  
281 deviations (Nieuwenhuis *et al.* 2012). Furthermore, we checked collinearity issues by deriving  
282 Variance Inflation Factors (VIF) (Fox and Weisberg 2019) of the standard linear model lacking  
283 the random effects. To test the significance of the predictors as a whole, we compared the fit  
284 of the full model with that of the null model comprising only the random factor and the  
285 intercept (Forstmeier and Schielzeth 2011).

286

#### 287 Ethical note

288 This study adhered to the Guidelines for the Treatment of Animals in Behavioral Research and  
289 Teaching (Animal Behaviour 2020) and the legal requirements of the country (France) in which  
290 the work was carried out. All the procedures were carried out in accordance with the  
291 European Communities Council Directive (86/609/EEC) and were done by authorized  
292 experimenters (license numbers 91-439 and 91-455 delivered by the departmental veterinary  
293 service). Animal housings were equipped with foliage, branches and several nest-boxes.  
294 Caloric restriction over a short period is physiologically sustainable for mouse lemurs and does  
295 not induce chronic stress (cortisol release), because mouse lemurs are adapted to drastic  
296 temporal changes in energy availability (Canale *et al.* 2011).

297

#### 298 Data availability statement

299 The datasets generated and analysed during the current study are not publicly available due  
300 to further analyses but are available from the corresponding author on reasonable request.

301

## 302 **Results**

303 In the AL condition, 8 individuals produced only grunts, 4 only tsaks and 1 both call types,  
304 whereas in the CR condition 4 individuals produced only grunts, 5 only tsaks and 3 both call  
305 types. The number of individuals giving either only one call type, tsaks or grunts, or both did  
306 not differ between the dietary regimes (Fisher exact test:  $p=0.32$ ). Individuals exposed to the  
307 CR diet had a lower body mass than those exposed to the AL diet (exact Mann Whitney U-test:  
308  $Z=1.32$ ,  $p<0.001$ , mean body mass  $\pm$  SD: CR=  $76.2 \pm 17.5$  g, AL= $99.6 \pm 10.4$  g).

309

310

311 *Grunts*

312 We analyzed 828 calls (1<sup>st</sup> element=467, 2<sup>nd</sup> element=361) produced by 9 AL and 7 CR  
 313 individuals. Measurements of acoustic variables are presented in (Table 3).

314

315 Table 3: Acoustic variables measured in grunts and tsaks in a study of mouse lemur  
 316 vocalizations housed at the CNRS, MNHN, Brunoy (2010).

Acoustic variables Mean ± SD	Grunts		Tsaks
	1 <sup>st</sup> element	2 <sup>nd</sup> element	
Duration (ms)	75.01 ± 24.23	106.13 ± 52.6	54.11 ± 19.44
Frequency range (kHz)	35.50 ± 11.07	14.69 ± 6.11	-
Mean peak frequency (kHz)	2.76 ± 3.82	0.74 ± 0.2	1.88 ± 2.84
2 <sup>nd</sup> dominant frequency band (kHz)	14.89 ± 2.27	13.66 ± 3.51	-
Mean lower frequency (kHz)	5.94 ± 4.41	1.04 ± 0.53	-
Mean central frequency (kHz)	13.68 ± 5.17	2.94 ± 1.92	-
Mean upper frequency (kHz)	22.16 ± 5.03	8.54 ± 4.02	-
Start lower frequency (kHz)	-	-	19.41 ± 2.79
End lower frequency (kHz)	-	-	18.67 ± 2.57
Mean lower frequency (kHz)	-	-	20.54 ± 2.66
Maximum lower frequency (kHz)	-	-	23.31 ± 3.28
Noise %	-	-	91.27 ± 11.61

317

318 The PCA extracted two principal components with Eigenvalues higher than 1, which  
 319 explained in total 85% of the variance (PC1=68%, PC2=17%). PC1 correlated most strongly with  
 320 variables characterizing the general energy distribution of the calls (Table 4), with higher  
 321 values of the PC1 characterizing calls that had a higher peak, lower, central, and upper  
 322 frequency and a broader frequency range. The 2<sup>nd</sup> dominant frequency band loaded most  
 323 strongly on PC2, with higher values characterizing higher pitched calls (Table 4).

324

325

326 Table 4: Loadings of each variable on the first (PC1) and second (PC2) principal component for  
 327 each acoustic variable measured in grunts and tsaks in a study of mouse lemur vocalizations  
 328 housed at the CNRS, MNHN, Brunoy (2010).

Acoustic variables	Grunts		Tsaks	
	PC1	PC2	PC1	PC2
Frequency range	0.48	-0.01	0.36	0.28
Peak frequency	0.36	-0.10	0.43	-0.19
2 <sup>nd</sup> dominant frequency band	0.03	0.99	-	-
Lower frequency	0.47	-0.08	-	-
Central frequency	0.48	0.01	0.48	0.06
Upper frequency	0.44	0.10		
Start central frequency	-	-	0.35	0.23
End central frequency	-	-	0.39	-0.27
Maximum central frequency	-	-	0.41	0.21
Noise	-	-	0.13	-0.84
% of variance explained	68	17	59	16

329

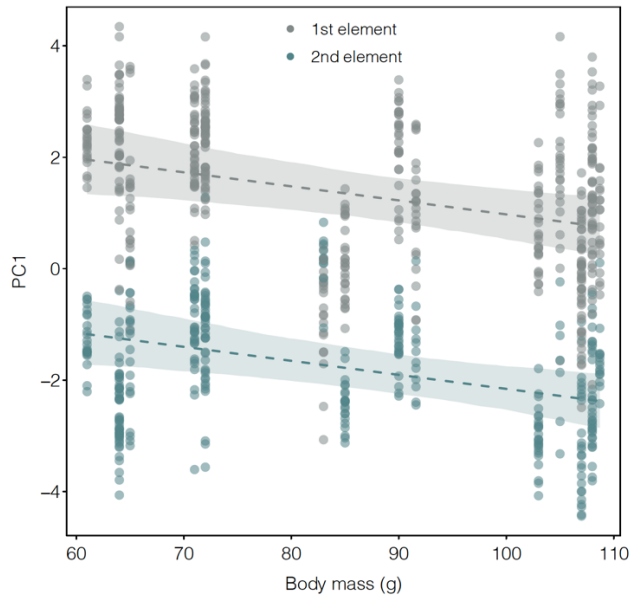
330

331 Overall, the model investigating variation in the PC1 of grunts with regard to body mass,  
 332 sex, and call element was significant (likelihood ratio test comparing full-null model  
 333 comparison:  $\chi^2=39.1$ ,  $df=3$   $p<0.001$ ). PC1 correlated negatively with body mass, with grunts  
 334 produced by heavier individuals having more energy in lower frequencies than calls produced  
 335 by lighter individuals (Table 4a, Fig 2). PC1 co-varied significantly with call element. The 1<sup>st</sup>  
 336 element of grunts had more energy in higher frequencies than the 2<sup>nd</sup> element.

337

338

339



340

341 Figure 2: Values of the PC1 (1<sup>st</sup> Principal Component) plotted against body mass for both call  
 342 elements of grunts recorded in a study of mouse lemur vocalizations housed at the CNRS,  
 343 MNHN, Brunoy (2010). Dashed lines indicate the regression lines and confidence intervals for  
 344 the 1<sup>st</sup> element in grey and the 2<sup>nd</sup> element in turquoise.

345

346 The model investigating the influence of body mass, sex and call element on variation of  
 347 the PC2 of grunts was also significant (likelihood ratio test comparing full-null model  
 348 comparison:  $\chi^2=10.8$ ,  $df=3$ ,  $p=0.013$ ). PC2 differed significantly between call elements, with  
 349 the 1<sup>st</sup> element containing more energy in higher frequencies than the 2<sup>nd</sup> element (Table 5b).  
 350 Variation in PC2 co-varied only by trend ( $p=0.07$ ) with body mass, and did not co-vary with sex  
 351 (Table 5b).

352 The model estimating the influence of diet, sex and call element on variation in PC1 was  
 353 significant (likelihood ratio test comparing full-null model comparison:  $\chi^2=37.7$ ,  $df=3$   $p<0.001$ ;  
 354 Table 5c). Diet and call element significantly influenced variation in PC1. AL individuals that  
 355 were heavier produced grunts that had lower PC1 values, and the 1<sup>st</sup> element had more energy  
 356 in higher frequencies than the 2<sup>nd</sup> element. The model estimating the influence of diet, sex  
 357 and call element on variation in PC2 was also significant (likelihood ratio test comparing full-  
 358 null model comparison:  $\chi^2=8.52$ ,  $df=3$   $p=0.037$ ; Table 5d). Call element but not diet or sex  
 359 influenced variation in PC2.

360

361



362 Table 5: Results of LMMs testing the influence of body mass, diet, call element and sex on  
 363 variation in PC1 and PC2 (1<sup>st</sup> and 2<sup>nd</sup> Principal Component; N=836; N<sub>ID</sub> = 16) of grunts in a study  
 364 of mouse lemur vocalizations housed at the CNRS, MNHN, Brunoy (2010). We tested body  
 365 mass and diet in separate models due to collinearity. Bold indicates p < 0.05.  
 366

Response variable	Term	Est	SE	P
a) PC1	Intercept	3.37	0.88	<sup>b</sup>
	Body mass	-0.03	0.01	<b>0.021</b>
	Call element (2nd) <sup>a</sup>	-3.02	0.31	<b>&lt;0.001</b>
	Sex (male) <sup>a</sup>	-0.26	0.36	0.488
b) PC2	Intercept	-1.01	0.65	<sup>b</sup>
	Body mass	0.02	0.01	0.063
	Call element (2nd) <sup>a</sup>	-0.50	0.16	<b>0.007</b>
	Sex (male) <sup>a</sup>	-0.27	0.28	0.347
c) PC1	Intercept	0.85	0.30	<sup>b</sup>
	Diet	0.81	0.34	<b>0.035</b>
	Call element (2nd) <sup>a</sup>	-3.02	0.31	<b>&lt;0.001</b>
	Sex (male) <sup>a</sup>	-0.03	0.37	0.943
d) PC1	Intercept	0.25	0.22	<sup>b</sup>
	Diet	0.08	0.27	0.768
	Call element (2nd) <sup>a</sup>	-0.51	0.16	<b>0.007</b>
	Sex (male) <sup>a</sup>	-0.16	0.29	0.598

367 <sup>a</sup> 1<sup>st</sup> element and females as reference categories

368 <sup>b</sup> Not shown as has no meaningful interpretation

369

### 370 *Tsaks*

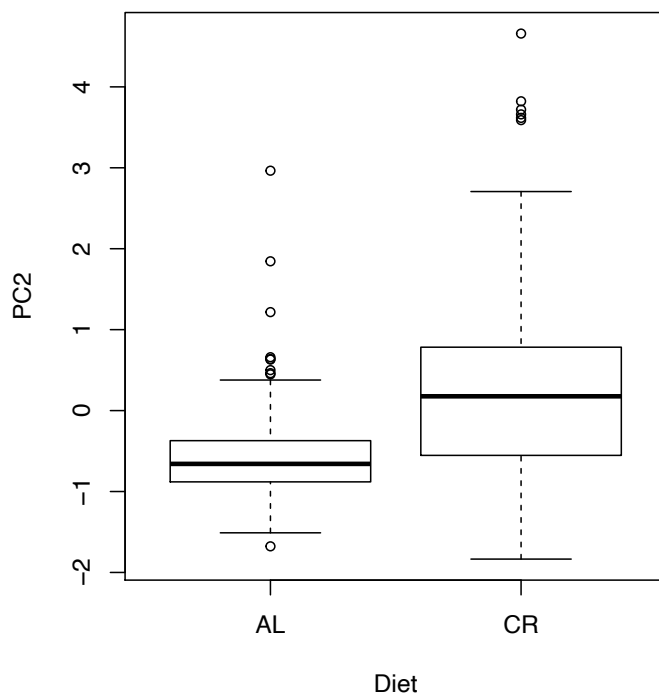
371 We analyzed 270 calls given by 5 AL and 8 CR individuals. Measurements of acoustic variables  
 372 are presented in (Table 3). The first two principal components of the PCA had Eigenvalues  
 373 higher than 1, which explained 75% of the variance in total (PC1=59%, PC2=16%). Similar to  
 374 grunts, the PC1 of tsaks correlated most strongly with variables characterizing the general  
 375 energy distribution within calls (Table 4), with higher values of the PC1 characterizing calls that  
 376 had a higher start, end, maximum, and mean lower frequency, a higher mean central and peak  
 377 frequency. PC2 loaded most strongly with noise, with higher values characterizing calls that  
 378 were less noisy (Table 4).

379 The models investigating the influence of body mass and sex on variation of the PC1 and  
 380 PC2 of tsaks were not significant (likelihood ratio test comparing full-null model comparison:  
 381 PC1:  $\chi^2=1.25$ , df=2 p=0.530, PC2:  $\chi^2=3.43$ , df=2, p=0.180; Table 6a, b). In the subset of calls  
 382 (N=83), for which we could measure the fundamental frequency, body mass did not correlate  
 383 with the mean fundamental frequency (Spearman rank, N=11, R=-0.24, p=0.484). This result

384 should be considered carefully, however, because we could only measure the fundamental  
385 frequency in one call in 3 of 11 individuals.

386 PC1 co-varied neither with diet or sex (likelihood ratio test comparing full-null model  
387 comparison:  $\chi^2=0.42$ ,  $df=2$   $p=0.810$ ; Table 6c). PC2, however, had lower values in tsaks given  
388 by AL individuals than by CR individuals (likelihood ratio test comparing full-null model  
389 comparison:  $\chi^2=10.70$ ,  $df=2$   $p=0.005$ ; Table 6d), suggesting that tsaks given by AL individuals  
390 were noisier than those given by CR individuals (Fig. 3).

391



392

393 Figure 3: PC2 (2<sup>nd</sup> Principal Component) of tsaks recorded in a study of mouse lemur  
394 vocalizations housed at the CNRS, MNHN, Brunoy (2010) as a function of dietary regimes  
395 (AL=ad libitum, CR= caloric restriction). Depicted are boxplots showing medians (solid lines),  
396 inter-quartile ranges (boxes), ranges (whiskers) and outliers (open circles) of PC2.

397

398

399

400

401

402 Table 6: Results of LMMs testing the influence of body mass, diet, call element and sex on  
 403 variation in PC1 and PC2 (1<sup>st</sup> and 2<sup>nd</sup> Principal Component; N=272; N<sub>ID</sub> = 15) of tsaks in a study  
 404 of mouse lemur vocalizations housed at the CNRS, MNHN, Brunoy (2010). We tested body  
 405 mass and diet in separate models due to collinearity. Bold indicates  $p < 0.05$ .

Response variable	Term	Est	SE	P
a) PC1	Intercept	-1.45	2.95	<sup>b</sup>
	Body mass	0.02	0.02	0.432
	Sex (male) <sup>a</sup>	-0.85	0.91	0.398
b) PC2	Intercept	0.04	0.68	<sup>b</sup>
	Body mass	-0.01	0.01	0.591
	Sex (male) <sup>a</sup>	0.58	0.34	0.112
c) PC1	Intercept	0.12	0.81	<sup>b</sup>
	Diet (CR) <sup>a</sup>	-0.11	0.93	0.906
	Sex (male) <sup>a</sup>	-0.46	0.91	0.621
d) PC2	Intercept	-0.67	0.21	<sup>b</sup>
	Diet (CR) <sup>a</sup>	0.68	0.24	<b>0.016</b>
	Sex (male) <sup>a</sup>	0.38	0.23	0.130

407 <sup>a</sup>sex and dietary regime: males and ad libitum (AL) as reference categories

408 <sup>b</sup>Not shown as has no meaningful interpretation

409

410

## 411 Discussion

412 We showed in this study, that mouse lemurs under caloric restriction had a lower body mass  
 413 than individuals under the *ad libitum* diet, suggesting that they were in poorer body condition  
 414 than individuals under the *ad libitum* diet. In grunts, the 1<sup>st</sup> element was characterized by  
 415 more energy in higher frequencies than the 2<sup>nd</sup> element. Body mass correlated negatively with  
 416 both principal components, with heavier individuals producing lower-frequency grunts.  
 417 Accordingly, AL individuals produced lower-frequency grunts than CR individuals. Acoustic  
 418 features of tsaks were predicted by neither body mass nor sex. However, tsaks produced by  
 419 AL individuals were noisier than those produced by CR individuals. Noisiness in vocalizations  
 420 has been suggested to be associated with aggressiveness, suggesting that mouse lemurs in  
 421 comparatively better condition might have been able to afford to be more aggressive. Hence,  
 422 manipulation of body condition via dietary regimes affected acoustic features of calls given  
 423 during aggression in different ways: acoustic features of grunts varied according to the rule of  
 424 acoustic allometry similar to what has been proposed for many other species. Acoustic  
 425 features of tsaks, however, varied according to motivational structural rules.

426

427 *Grunts*

428 Grunts consist of two elements that differ in distribution of energy, with the 1<sup>st</sup> element having  
429 more energy in higher frequencies than the 2<sup>nd</sup> element. Body mass correlated negatively with  
430 the distribution of energy in both elements, with heavier individuals producing lower-  
431 frequency grunts. These results are in line with the rule for acoustic allometry, supporting  
432 earlier studies in many other species (Bowling *et al.* 2017; Charlton and Reby 2016; Fitch and  
433 Hauser 1995; Garcia *et al.* 2018; Gingras *et al.* 2013; Hauser 1993; Wallschläger 1980).  
434 Although many studies have focused on fundamental frequency or formant dispersion to  
435 assess acoustic allometry (Fitch 1997; Fitch and Hauser 1995; Fitch and Reby 2001), a more  
436 recent comparative study revealed that the frequency with the highest amplitude also co-  
437 varies with body mass in carnivores and primates (Bowling *et al.* 2017). Here, we show that a  
438 general shift in frequencies, summarized as principal components, also co-varies with body  
439 mass in mouse lemur grunts.

440 In grey mouse lemurs in the wild, body mass predicts several fitness proxies such as  
441 survival (Hämäläinen *et al.* 2014; Rakotoniaina *et al.* 2017), the ability to cope better with  
442 environmental constraints through physiological mechanisms, such as torpor (Vuarin *et al.*  
443 2013) or allostatic load in males (Hämäläinen *et al.* 2015), reproductive success in males  
444 (Eberle and Kappeler 2004b) and the relative proportion of polyandrous mating in females in  
445 captivity (Huchard *et al.* 2012). Hence, acoustic features that convey information about body  
446 mass might serve as honest signals (Zahavi 1977). Similarly, call rates of mouse lemur trills that  
447 are produced in the mating season to attract mates have been suggested to be associated  
448 with rank and to advertise quality (Zimmerman 1996). Hence, calling rates of advertisement  
449 calls and acoustic features of aggressive grunts may serve to advertise quality in mouse  
450 lemurs.

451 Mouse lemurs may benefit from signaling their current body condition during agonistic  
452 interactions, in particular. During the mating season, males roam widely in search of receptive  
453 females by enlarging their home ranges to cover the center of activity of up to 21 females  
454 (Eberle and Kappeler, 2004a). Once they encounter an estrous female, they attempt to mate  
455 guard her by staying close to her and trying to fend off rivals. An estrous female is usually  
456 guarded by several males, and the longer a male can guard a female, the fewer mating  
457 partners she has. During mate guarding, males fight with several other males and male

458 mortality increases during the brief mating season because of this risky mating strategy (Kraus  
459 *et al.* 2008). In addition, in captivity females preferentially accepted copulations from more  
460 competitive, aggressive males (Gomez *et al.* 2012). Hence, males clearly benefit from signaling  
461 condition and fighting ability in vocalizations both during agonistic interactions with rivals and  
462 to obtain access to matings.

463 In contrast, females may face male harassment during the mating season. They are  
464 receptive for only one night, but males harass and inspect female's reproductive state over  
465 the whole mating season (Eberle and Kappeler 2004a). In addition, estrous females are  
466 guarded by several males but may try to escape guarding males (Eberle and Kappeler 2004a)  
467 to mate with other males (Huchard *et al.* 2012). Hence, signaling their condition during such  
468 conflicts might also be beneficial for females. Outside the mating season, both males and  
469 females produce grunts during aggressive interactions and during disturbances at the sleeping  
470 site (Radespiel and Zimmermann 2001, Zimmermann 2010). In both contexts individuals might  
471 benefit from signaling condition and fighting ability. Playback experiments are now required  
472 to determine whether mouse lemurs also respond to acoustic cues indicating differently sized  
473 callers, as in other species (Reby *et al.* 2005; Charlton *et al.* 2010b).

474

#### 475 *Tsaks*

476 In contrast to grunts, acoustic features of tsaks did not correlate with body mass. Neither shifts  
477 in the general energy distribution nor the mean fundamental frequency co-varied with body  
478 mass. Tsaks have a relatively a narrow frequency bandwidth with little variation across calls.  
479 Their mean central frequency, for example, varied on average only by about 2 kHz, whereas  
480 the mean central frequency in grunts varied by about 5 kHz. Hence, the production of tsaks  
481 may be too constrained to co-vary with changes in body mass. Unfortunately, we could only  
482 measure the fundamental frequency in a subset of calls, and the lack of a significant  
483 correlation between fundamental frequency and body mass might be due to the small sample  
484 size. Additional recordings are required to understand whether acoustic features of tsaks co-  
485 vary principally with body mass, for example during the development from infancy to  
486 adulthood.

487 Dietary treatment predicted the amount of noisy energy in tsaks. Individuals on the *ad*  
488 *libitum* diet produced noisier calls than caloric restricted individuals. According to the  
489 motivational-structural rules (Morton 1977), noisiness is associated with aggression. More

490 aggressive calls are characterized by noisier acoustic structure in squirrel monkeys (*Saimiri*  
491 *sciureus*) and Geoffroy's spider monkeys (*Ateles geoffroyi*) (Fichtel *et al.* 2001; Ordóñez-Gómez  
492 *et al.* 2015). Since mouse lemurs on the *ad libitum* dietary regime were in better condition,  
493 they might have been able to afford to be more aggressive and hence produced more noisy  
494 calls. Moreover, in another study including some females that also participated in this study,  
495 caloric restricted females mated only with one male, whereas females on the *ad libitum*  
496 dietary regime mated with several males (Huchard *et al.* 2012). Since mating is costly and body  
497 mass loss during mating correlated with the number of mating partners (Huchard *et al.* 2012),  
498 caloric restricted females might be less likely to engage in energetic costly aggressive behavior  
499 during disturbances.

500       Vocalizations that contain more nonlinear dynamics, such as noise, have been suggested  
501 to be more unpredictable, and this unpredictability may function to prevent receivers from  
502 ignoring such calls (Fitch and Hauser 1995; Fitch *et al.* 2002). Playback experiments revealed  
503 that yellow-bellied marmots (*Marmota flaviventris*) responded more strongly to alarm calls  
504 including noise than to control calls, supporting the notion that the adaptive value of non-  
505 linearities might be to prevent habituation (Blumstein and Récapet 2008). Again, playback  
506 experiments are required to examine whether mouse lemurs also respond more strongly to  
507 noisier tsaks. In principle, signaling condition or the motivational state is beneficial both during  
508 competition over mates and to defend resources such as feeding or sleeping sites or to deter  
509 predators, contexts in which both call types are produced (Eberle and Kappeler 2008; Rahlfs  
510 and Fichtel 2010; Zimmermann 2010).

511       Finally, variation in acoustic features can also be due to group membership or relatedness  
512 (Mitani *et al.* 1992; Levréro *et al.* 2015). However, we think it is unlikely that group  
513 membership or relatedness explains variation in acoustic features across dietary regimes  
514 because only a few dyads of mouse lemurs were closely related in our study, and individuals  
515 were either housed individually or we recorded only individuals from different groups if they  
516 were housed in groups.

517       In conclusion, manipulation of body condition via dietary regimes affected acoustic  
518 features of calls given during aggression in different ways: mouse lemurs in better condition  
519 produced lower pitched grunts than those in weaker condition, supporting the rule of acoustic  
520 allometry. In contrast, the acoustic features of tsaks did not support the rule of acoustic  
521 allometry but did follow motivational structural rules. Mouse lemurs in better condition might

522 have been able to show more aggression and produced more noisy calls. However, we only  
523 compared vocalizations of different individuals in which body mass was manipulated via  
524 caloric restriction. Since mouse lemurs undergo seasonal changes of up one third of their body  
525 mass in their natural habitat (Hämäläinen *et al.* 2014), a longitudinal study is needed to  
526 examine how much changes in body mass within individuals are also reflected in the acoustic  
527 structure of aggressive vocalizations. This would also allow us to investigate whether acoustic  
528 cues indicate stable, long-term attributes of callers as well as more flexible variation in  
529 condition and, hence, fighting ability.

530

531

### 532 **Acknowledgements**

533 This paper is dedicated to Elke Zimmermann, a pioneer in the study of communication in  
534 nocturnal primates. We are very grateful to Ute Radespiel and Marina Scheumann for the  
535 invitation to contribute to the Festschrift for Elke Zimmermann. Financial support was  
536 obtained from UMR 7179 CNRS MNHN, and a PEPS 2009 grant attributed to P.Y.H. by INEE-  
537 CNRS. E.H. was funded by a Deutsches Forschungsgemeinschaft (DFG) research grant (no HU  
538 1820/1-1). Publication ISEM n°2021-267.

539

540 **Author Contributions:** CF conceived, designed, and executed this study. MP, EH, P-YH were  
541 responsible for animal housing and conducting the caloric restriction experiment. CF and PMK  
542 wrote the manuscript; other authors provided editorial advice.

543

544 **Conflict of Interest**

545 The authors declare that they have no conflict of interest.

546

547

### 548 **References**

549 Animal Behaviour (2020). Guidelines for the treatment of animals in behavioural research  
550 and teaching. *Animal Behaviour*, 159, I-XI. <https://doi.org/10.1006/anbe.1996.0293>  
551 Arnott, G., Elwood, R. W. (2009). Assessment of fighting ability in animal contests. *Animal*  
552 *Behaviour*, 77, 991-1004. <https://doi.org/10.1016/j.anbehav.2009.02.010>  
553 Aujard, F., Perret, M. & Vanier, G. (1998). Thermoregulatory responses to variations of  
554 photoperiod and ambient temperature in the lesser mouse lemur: a primitive or an  
555 advanced adaptive character? *Journal of Comparative Physiology B*, 168, 540–548.  
556 <https://link.springer.com/article/10.1007/s003600050175>

557 Barelli, C., Mundry, R., Heistermann, M., & Hammerschmidt, K. (2013). Cues to androgens  
558 and quality in male gibbon songs. *PLoS ONE*, *8*, e82748–9.  
559 <https://doi.org/10.1371/journal.pone.0082748>

560 Barr, D. J., Levy, R., Scheepers, C., & Tily, H. J. (2013). Random effects structure for  
561 confirmatory hypothesis testing: Keep it maximal. *Journal of Memory and Language*, *68*,  
562 255–278. <https://doi.org/10.1016/j.jml.2012.11.001>

563 Bates D, Mächler M, Bolker B, Walker S (2015). Fitting linear mixed-effects models using  
564 lme4. *Journal of Statistical Software*, *67*, 1–48.  
565 <https://www.jstatsoft.org/article/view/v067i01>

566 Blumstein, D. T., & Récapet, C. (2009). The sound of arousal: the addition of novel non-  
567 linearities increases responsiveness in marmot alarm calls. *Ethology*, *115*, 1074–1081.  
568 <https://doi.org/10.1111/j.1439-0310.2009.01691.x>

569 Bowling, D. L., Garcia, M., Dunn, J. C., Ruprecht, R., Stewart, A., Frommolt, K. H., & Fitch, W.  
570 T. (2017). Body size and vocalization in primates and carnivores. *Scientific Reports*, *7*,  
571 41070. <https://www.nature.com/articles/srep41070>

572 Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication*.  
573 2nd. Sunderland, Massachusetts: Sinauer.

574 Buesching, C. D., Heistermann, M., Hodges, J.K. & Zimmermann E. (1998). Multimodal  
575 oestrus advertisement in a small nocturnal prosimian, *Microcebus murinus*. *Folia*  
576 *Primatologica*, *69*, 295–308. <https://doi.org/10.1159/000052718>

577 Canale, C. I., Perret, M., Thiéry, M. & Henry, P.-Y. (2011). Physiological flexibility and  
578 acclimation to food shortage in a heterothermic primate. *Journal of Experimental Biology*,  
579 *214*, 551–560. <https://jeb.biologists.org/content/214/4/551>

580 Chajewski, M. (2009). rela: Scale item analysis. R package version 4.1.

581 Charlton, B. D., Zhihe, Z. & Snyder, R. J. (2009). The information content of giant panda,  
582 *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. *Animal Behaviour*, *78*,  
583 893–898. <https://doi.org/10.1016/j.anbehav.2009.06.029>

584 Charlton, B. D., Keating, J. L., Rengui, L., Huang, Y., & Swaisgood, R. R. (2010a). Female giant  
585 panda (*Ailuropoda melanoleuca*) chirps advertise the caller's fertile phase. *Proceedings of*  
586 *the Royal Society B: Biological Sciences*, *277*, 1101–1106.  
587 <https://doi.org/10.1098/rspb.2009.1431>

588 Charlton, B. D., Zhihe, Z., & Snyder, R. J. (2010b). Giant pandas perceive and attend to  
589 formant frequency variation in male bleats. *Animal Behaviour*, *79*, 1221–1227.  
590 <https://doi.org/10.1016/j.anbehav.2010.02.018>

591 Charlton, B. D. & Reby, D. (2016). The evolution of acoustic size exaggeration in terrestrial  
592 mammals. *Nature Communication*, *7*, 12739, <https://doi.org/10.1038/ncomms12739>

593 Charlton, B. D., Pisanski, K., Raine, J., & Reby, D. (2020). Coding of static information in  
594 terrestrial mammal vocal signals. In *Coding Strategies in Vertebrate Acoustic*  
595 *Communication* (Vol. 7, pp. 115–136). Cham: Springer International Publishing.

596 Seyfarth, R. M., & Cheney, D. L. (2003). Signalers and receivers in animal  
597 communication. *Annual Review of Psychology*, *54*, 145-173.

598 Clarke, E., Reichard, U. H., Zuberbühler, K., & Emery, N. (2006). The syntax and meaning of  
599 wild gibbon songs. *PLoS ONE*, *1*, e73. <https://doi.org/10.1371/journal.pone.0000073>

600 Dal-Pan, A., Terrien, J., Pifferi, F., Botalla, R., Hardy, I., Marchal, J., Zahariev, A., Chery, I.,  
601 Zizzari, P., Perret, M., Picq, J. L., Epelbaum, J., Blanc, S. & Aujard, F. (2011). Caloric  
602 restriction or resveratrol supplementation and ageing in a non-human primate: first-year  
603 outcome of the RESTRIKAL study in *Microcebus murinus*. *Age (Dordrecht, Netherlands)*,  
604 *33*, 15–31. DOI: [10.1007/s11357-010-9156-6](https://doi.org/10.1007/s11357-010-9156-6)



605 Dammhahn, M., & Kappeler, P. M. (2008). Small-scale coexistence of two mouse lemur  
606 species (*Microcebus berthae* and *M. murinus*) within a homogeneous competitive  
607 environment. *Oecologia*, *157*, 473–483. <https://dx.doi.org/10.1007%2Fs00442-008-1079->  
608 [x](https://dx.doi.org/10.1007%2Fs00442-008-1079-x)

609 Dewar, R. E., & Richard, A. F. (2007). Evolution in the hypervariable environment of  
610 Madagascar. *Proceedings of the National Academy of Sciences of the United States of*  
611 *America*, *104*, 13723–13727. <https://doi.org/10.1073/pnas.0704346104>

612 Dunn, J. C., Halenar, L. B., Davies, T. G., Cristobal-Azkarate, J., Reby, D., Sykes, D., Degg, S.,  
613 Fitch, W.T., Knapp, L. A. (2015). Evolutionary trade-off between vocal tract and testes  
614 dimensions in howler monkeys. *Current Biology*, *25*, 2839-2844.  
615 <https://www.sciencedirect.com/science/article/pii/S0960982215011094>

616 Gomez, D., Huchard, E., Henry, P.-Y., & Perret, M. (2012). Mutual mate choice in a female-  
617 dominant and sexually monomorphic primate. *American Journal of Physical*  
618 *Anthropology*, (147), 370–379. <https://doi.org/10.1002/ajpa.21653>

619 Eberle, M., & Kappeler, P. M. (2004a). Selected polyandry: female choice and inter-sexual  
620 conflict in a small nocturnal solitary primate (*Microcebus murinus*). *Behavioral Ecology*  
621 *and Sociobiology*, *57*, 91–100. <https://link.springer.com/article/10.1007/s00265-004->  
622 [0823-4](https://link.springer.com/article/10.1007/s00265-004-0823-4)

623 Eberle, M., & Kappeler, P. M. (2004b). Sex in the dark: determinants and consequences of  
624 mixed male mating tactics in *Microcebus murinus*, a small solitary nocturnal primate.  
625 *Behavioral Ecology and Sociobiology*, *57*, 77–90.  
626 <https://link.springer.com/article/10.1007/s00265-004-0826-1>

627 Eberle, M., & Kappeler, P. M. (2008). Mutualism, reciprocity, or kin selection? Cooperative  
628 rescue of a conspecific from a boa in a nocturnal solitary forager the gray mouse lemur.  
629 *American Journal of Primatology*, *70*, 410–414. <https://doi.org/10.1002/ajp.20496>

630 Ey, E., Hammerschmidt, K., Seyfarth, R. M., & Fischer, J. (2007). Age- and sex-related  
631 variations in clear calls of *Papio ursinus*. *International Journal of Primatology*, *28*(4), 947–  
632 960. <https://link.springer.com/article/10.1007/s10764-007-9139-3>

633 Fichtel, C. (2014). Acoustic differences in loud calls of Decken’s and Crowned sifakas  
634 (*Propithecus deckenii* and *P. coronatus*) at two sites in Madagascar, 1–7.  
635 <https://doi.org/10.1896/052.028.0105>

636 Fichtel, C., Hammerschmidt, K., & Jürgens, U. (2001). On the expression of emotion. A multi-  
637 parametric analysis of different states of aversion in the squirrel monkey. *Behaviour*, *138*,  
638 97-116. <https://doi.org/10.1163/15685390151067094>

639 Fichtel, C., & Hammerschmidt, K. (2002). Responses of redfronted lemurs to experimentally  
640 modified alarm calls: evidence for urgency-based changes in call structure. *Ethology*, *108*,  
641 763–777. <https://doi.org/10.1046/j.1439-0310.2002.00816.x>

642 Fichtel, C., & Hammerschmidt, K. (2003). Responses of squirrel monkeys to their  
643 experimentally modified mobbing calls. *The Journal of the Acoustical Society of America*,  
644 *113*(5), 2927–2932. <https://doi.org/10.1121/1.1548158>

645 Fichtel, C., Perry, S., & Gros Louis, J. (2005). Alarm calls of white-faced capuchin monkeys: an  
646 acoustic analysis. *Animal Behaviour*, *70*, 165–176.  
647 <https://doi.org/10.1016/j.anbehav.2004.09.020>

648 Fischer, J., Kitchen, D. M., Seyfarth, R. M. & Cheney, D. L. (2004). Baboon loud calls advertise  
649 male quality: acoustic features and their relation to rank, age, and exhaustion. *Behavioral*  
650 *Ecology and Sociobiology*, *56*, 140–148. <https://link.springer.com/article/10.1007/s00265->  
651 [003-0739-4](https://link.springer.com/article/10.1007/s00265-003-0739-4)

652 Fitch, W. (1997). Vocal tract length and formant frequency dispersion correlate with body  
653 size in rhesus macaques. *The Journal of the Acoustical Society of America*, *102*, 1213.  
654 <https://doi.org/10.1121/1.421048>

655 Fitch, W. T. & Hauser, M. D (1995) Vocal production in nonhuman primates: acoustics,  
656 physiology, and functional constraints on “honest” advertisement. *American Journal of*  
657 *Primatology*, *37*, <https://doi.org/10.1002/ajp.1350370303>

658 Fitch, T. W., & Reby, D. (2001). The descended larynx is not uniquely human. *Proceedings of*  
659 *the Royal Society of London. Series B: Biological Sciences*, *268*, 1669-1675.  
660 <https://doi.org/10.1098/rspb.2001.1704>

661 Fitch, W. T., Neubauer, J. & Herzog, H. (2002). Calls out of chaos: the adaptive significance of  
662 nonlinear phenomena in mammalian vocal production. *Animal Behaviour*, *63*, 407–418.  
663 <https://doi.org/10.1006/anbe.2001.1912>

664 Forstmeier, W. Schielzeth, H. (2011). Cryptic multiple hypotheses testing in linear models:  
665 overestimated effect sizes and the winner’s curse. *Behavioral Ecology & Sociobiology*, *65*,  
666 47–55. <https://doi.org/10.1007/s00265-010-1038-5>

667 Fox J, Weisberg S (2019). *An R Companion to Applied Regression*, Third edition. Sage,  
668 Thousand Oaks CA <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.

669 Garcia, M., Herbst, C. T., Bowling, D. L., Dunn, J. C., & Fitch, W. T. (2018). Acoustic allometry  
670 revisited: morphological determinants of fundamental frequency in primate vocal  
671 production, *Scientific Reports*, *7*, 10450. [https://www.nature.com/articles/s41598-017-](https://www.nature.com/articles/s41598-017-11000-x)  
672 [11000-x](https://www.nature.com/articles/s41598-017-11000-x)

673 Gingras, B., Boeckle, M., Herbst, C. T., & Fitch, W. T. (2013). Call acoustics reflect body size  
674 across four clades of anurans. *Journal of Zoology*, *289*, 143-  
675 150. <https://doi.org/10.1111/j.1469-7998.2012.00973.x>

676 Giroud, S., Blanc, S., Aujard, F., Bertrand, F., Gilbert, C. & Perret, M. (2008). Chronic food  
677 shortage and seasonal modulations of daily torpor and locomotor activity in the grey  
678 mouse lemur (*Microcebus murinus*). *American Journal of Physiology Regulatory*  
679 *Integrative and Comparative Physiology*, *294*, R1958–R1967.  
680 <https://doi.org/10.1152/ajpregu.00794.2007>

681 Gomez, D., Huchard, E., Henry, P.-Y., & Perret, M. (2012) Mutual mate choice in a female-  
682 dominant and sexually monomorphic primate. *American Journal of Physical*  
683 *Anthropology*, *147*, 370–379. <https://doi.org/10.1002/ajpa.21653>

684 Hauser, M. D. (1993). The evolution of nonhuman primate vocalizations: effects of  
685 phylogeny, body weight, and social context. *The American Naturalist*, *142*, 528–542.  
686 <https://doi.org/10.1086/285553>

687 Hämäläinen, A., Dammhahn, M., Aujard, F., Eberle, M., Hardy, I., Kappeler, P. M., Perret, M.,  
688 Schliehe-Diecks, S. & Kraus, C. (2014). Senescence or selective disappearance? Age  
689 trajectories of body mass in wild and captive populations of a small-bodied  
690 primate. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140830.  
691 <https://doi.org/10.1098/rspb.2014.0830>

692 Hämäläinen, A., Heistermann, M., & Kraus, C. (2015). The stress of growing old: sex-and  
693 season-specific effects of age on allostatic load in wild grey mouse  
694 lemurs. *Oecologia*, *178*, 1063-1075. DOI: [10.1007/s00442-015-3297-3](https://doi.org/10.1007/s00442-015-3297-3)

695 Huchard, E., Canale, C. I., Le Gros, C., Perret, M., Henry, P.-Y. & Kappeler, P. M. (2012).  
696 Convenience polyandry or convenience polygyny? Costly sex under female control in a  
697 promiscuous primate. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 1371–  
698 1379. <https://doi.org/10.1098/rspb.2011.1326>

699 Jennions, M. D. & Petrie, M. (2000). Why do females mate multiply? A review of the genetic  
700 benefits. *Biological Reviews*, 75, 21–64. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-185X.1999.tb00040.x)  
701 [185X.1999.tb00040.x](https://doi.org/10.1111/j.1469-185X.1999.tb00040.x)

702 Jonart, L. M., Hill, G. E., & Badyaev, A. V. (2007). Fighting ability and motivation:  
703 determinants of dominance and contest strategies in females of a passerine bird. *Animal*  
704 *Behaviour*, 74(6), 1675-1681  
705 <https://www.sciencedirect.com/science/article/abs/pii/S0003347207003144>

706 Kitchen, D., Seyfarth, R., Fischer, J., & Cheney, D. (2003). Loud calls as indicators of  
707 dominance in male baboons (*Papio cynocephalus ursinus*). *Behavioral Ecology and*  
708 *Sociobiology*, 53, 374-385. <https://doi.org/10.1007/s00265-003-0588-1>

709 Kraus, C., Eberle, M., & Kappeler, P. M. (2008). The costs of risky male behaviour: sex  
710 differences in seasonal survival in a small sexually monomorphic primate. *Proceedings of*  
711 *the Royal Society London B*, 275, 1635–1644. <https://doi.org/10.1098/rspb.2008.0200>

712 Kuznetsova A., Brockhoff P.B., & Christensen, R.H.B. (2017). “lmerTest Package: Tests in  
713 Linear Mixed Effects Models.” *Journal of Statistical Software*, 82, 1–26.  
714 [https://doi: 10.18637/jss.v082.i13](https://doi.org/10.18637/jss.v082.i13).

715 Leliveld, L M., Scheumann, M., & Zimmermann, E. (2011). Acoustic correlates of individuality  
716 in the vocal repertoire of a nocturnal primate (*Microcebus murinus*). *The Journal of the*  
717 *Acoustical Society of America*, 129, 2278-2288. <https://doi.org/10.1121/1.3559680>

718 Levréro, F., Carrete-Vega, G., Herbert, A., Lawabi, I., Courtiol, A., Willaume, E., Kappeler,  
719 P.M., Charpentier, M. J. E. (2015). Social shaping of voices does not impair phenotype  
720 matching of kinship in mandrills. *Nature Communications*, 6, 1-7.  
721 <https://www.nature.com/articles/ncomms8609?origin=ppub>

722 Malalaharivony HS, Fichtel C, Heistermann M, Kappeler PM (2021) Maternal stress effects on  
723 infant development in wild Verreaux's sifaka (*Propithecus verreauxi*). *Behavioral Ecology*  
724 *& Sociobiology* 75: 143

725 Manser, M. (2001). The acoustic structure of suricates' alarm calls varies with predator type  
726 and the level of response urgency. *Proceedings of the Royal Society London B*: 268: 2315–  
727 2324. <https://doi.org/10.1098/rspb.2001.1773>

728 Masters, W. M., Raver, K. A. S. & Kazial, K. A. (1995). Sonar signals of big brown bats,  
729 *Eptesicus fuscus*, contain information about individual identity, age and family affiliation.  
730 *Animal Behaviour*, 50, 1243–1260. [https://doi.org/10.1016/0003-3472\(95\)80041-7](https://doi.org/10.1016/0003-3472(95)80041-7)

731 Mitani, J. C., Hasegawa, T., Gros-Louis, J., Marler, P., Byrne, R. (1992). Dialects in wild  
732 chimpanzees? *American Journal of Primatology*, 27, 233-  
733 243. <https://doi.org/10.1002/ajp.1350270402>

734 Morton, E. S. (1977). On the occurrence and significance of motivation-structural rules in  
735 some bird and mammal sounds. *American Naturalist*, 111, 855–869.  
736 <https://www.jstor.org/stable/2460385>

737 Nieuwenhuis, R., te Grotenhuis, M., and Pelzer, B. (2012). Influence.me: Tools for detecting  
738 influential data in mixed effects models. *The R Journal*, 4:38–47. [https://journal.r-](https://journal.r-project.org/archive/2012/RJ-2012-011/index.html)  
739 [project.org/archive/2012/RJ-2012-011/index.html](https://journal.r-project.org/archive/2012/RJ-2012-011/index.html)

740 Ordóñez-Gómez, J. D., Dunn, J. C., Arroyo-Rodríguez, V., Méndez-Cárdenas, M. G., Márquez-  
741 Arias, A., & Santillán-Doherty, A. M. (2015). Role of emitter and severity of aggression  
742 influence the agonistic vocalizations of Geoffroy's spider monkeys (*Ateles geoffroyi*).  
743 *International Journal of Primatology*, 36(2), 429–440.  
744 <https://link.springer.com/article/10.1007/s10764-015-9833-5>

745 Perneger, T. V. (1998). What's wrong with Bonferroni adjustments. *Bmj*, 316(7139), 1236-  
746 1238. <https://doi.org/10.1136/bmj.316.7139.1236>

747 Perret, M. & Aujard, F. (2001). Regulation by photoperiod of seasonal changes in body mass  
748 and reproductive function in gray mouse lemurs (*Microcebus murinus*): differential  
749 responses by sex. *International Journal of Primatology*, 22, 5–24.  
750 <https://doi.org/10.1023/A:1026457813626>

751 Puts, D. A., Hill, A. K., Bailey, D. H., Walker, R. S., Rendall, D., Wheatley, J. R., et al. (2016).  
752 Sexual selection on male vocal fundamental frequency in humans and other anthropoids.  
753 *Proceedings of the Royal Society London B*, 283, 20152830–8.  
754 <https://doi.org/10.1098/rspb.2015.2830>

755 Queen, J. P., Quinn, G. P., & Keough, M. J. (2002). *Experimental design and data analysis for*  
756 *biologists*. Cambridge university press.

757 R Core Team. (2020). *R: A Language and Environment for Statistical Computing*. R  
758 Foundation for Statistical Computing. Vienna, Austria.

759 Radespiel, U. (2000). Sociality in the gray mouse lemur (*Microcebus murinus*) in  
760 northwestern Madagascar. *American Journal of Primatology*, 51, 21–40.  
761 [https://doi.org/10.1002/\(SICI\)1098-2345\(200005\)51:1<21::AID-AJP3>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1098-2345(200005)51:1<21::AID-AJP3>3.0.CO;2-C)

762 Radespiel, U., & Zimmermann, E. (2001). Female dominance in captive gray mouse lemurs  
763 (*Microcebus murinus*). *American Journal of Primatology*, 54, 181–192.  
764 <https://doi.org/10.1002/ajp.1029>

765 Rahlfs, M. & Fichtel, C. (2010). Anti-Predator Behaviour in a Nocturnal Primate, the Grey  
766 Mouse Lemur (*Microcebus murinus*). *Ethology*, 116, 429–439.  
767 <https://doi.org/10.1111/j.1439-0310.2010.01756.x>

768 Rakotoniaina, J. H., Kappeler, P. M., Kaesler, E., Hämäläinen, A. M., Kirschbaum, C., & Kraus,  
769 C. (2017). Hair cortisol concentrations correlate negatively with survival in a wild primate  
770 population. *BMC ecology*, 1–13. <https://doi.org/10.1186/s12898-017-0140-1>

771 Reby, D. & McComb, K. (2003). Anatomical constraints generate honesty: acoustic cues to  
772 age and weight in the roars of red deer stags. *Animal Behaviour*, 65, 519–530.  
773 <https://doi.org/10.1006/anbe.2003.2078>

774 Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W., & Clutton-Brock, T. (2005). Red  
775 deer stags use formants as assessment cues during intrasexual agonistic interactions.  
776 *Proceedings of the Royal Society London B*, 272, 941.  
777 <https://doi.org/10.1098/rspb.2004.2954>

778 Rendall, D., Rodman, P. S. & Emond, R. E. (1996). Vocal recognition of individuals and kin in  
779 free-ranging rhesus monkeys. *Animal Behaviour*, 51, 1007–1015.  
780 <https://doi.org/10.1006/anbe.1996.0103>

781 Ryan, M. J. & Brenowitz, E. A. (1985). The role of body size, phylogeny, and ambient noise in  
782 the evolution of bird song. *American Naturalist*, 87–100.  
783 <https://www.jstor.org/stable/2461564>

784 Scheumann, M., Zimmermann, E., & Deichsel, G. (2007). Context-specific calls signal infants'  
785 needs in a strepsirrhine primate, the gray mouse lemur (*Microcebus murinus*).  
786 *Developmental Psychobiology*, 49, 708–718. <https://doi.org/10.1002/dev.20234>

787 Schielzeth, H. & Forstmeier, W. 2009. Conclusions beyond support: overconfident estimates  
788 in mixed models. *Behavioral Ecology*, 20, 416–420.  
789 <https://doi.org/10.1093/beheco/arn145>

790 Schmid, J. & Kappeler, P. M. (1998). Fluctuating sexual dimorphism and differential  
791 hibernation by sex in a primate, the gray mouse lemur (*Microcebus murinus*). *Behavioral*  
792 *Ecology and Sociobiology*, 43, 125–132. <https://doi.org/10.1007/s002650050474>

793 Schrader, L., & Hammerschmidt, K. (1997). Computer-aided analysis of acoustic parameters  
794 in animal vocalisations: a multi-parametric approach. *Bioacoustics*, 7, 247-265.  
795 <https://doi.org/10.1080/09524622.1997.9753338>

796 Semple, S., McComb, K., Alberts, S., & Altmann, J. (2002). Information content of female  
797 copulation calls in yellow baboons. *American Journal of Primatology*, 56, 43–56.  
798 <https://doi.org/10.1002/ajp.1062>

799 Taylor, A. & Reby, D. (2010). The contribution of source–filter theory to mammal vocal  
800 communication research. *Journal of Zoology*, 280, 221–236.  
801 <https://doi.org/10.1111/j.1469-7998.2009.00661.x>

802 Vannoni, E. & McElligott, A. G. (2008). Low frequency groans indicate larger and more  
803 dominant fallow deer (*Dama dama*) males. *PLoS One*, 3, e3113.  
804 <https://doi.org/10.1371/journal.pone.0003113>

805 Vieira, M. C., & Peixoto, P. E. (2013). Winners and losers: a meta-analysis of functional  
806 determinants of fighting ability in arthropod contests. *Functional Ecology*, 27(2), 305-313.

807 Vuarin, P., Dammhahn, M., & Henry, P.-Y. (2013). Individual flexibility in energy saving: body  
808 size and condition constrain torpor use. *Functional Ecology*, 27, 793-799.  
809 <https://doi.org/10.1111/1365-2435.12069>

810 Wallschläger, D. (1980). Correlation of song frequency and body weight in passerine birds.  
811 *Experientia*, 36, 412. <https://doi.org/10.1007/BF01975119>

812 Zahavi, A. (1977). The cost of honesty (further remarks on the handicap principle). *Journal of*  
813 *Theoretical Biology*, 67, 603–605. DOI: [10.1016/0022-5193\(77\)90061-3](https://doi.org/10.1016/0022-5193(77)90061-3)

814 Zimmermann, E. 1996. Castration affects the emission of an ultrasonic vocalization in a  
815 nocturnal primate, the grey mouse lemur (*Microcebus murinus*). *Physiology & Behavior*,  
816 60, 693-697. DOI: [10.1016/0031-9384\(96\)81674-x](https://doi.org/10.1016/0031-9384(96)81674-x)

817 Zimmermann, E., Vorobieva, E., Wrogemann, D., & Hafen, T. (2000). Use of vocal  
818 fingerprinting for specific discrimination of gray (*Microcebus murinus*) and rufous mouse  
819 lemurs (*Microcebus rufus*). *International Journal of Primatology*, 21, 837–852.  
820 <https://doi.org/10.1023/A:1005594625841>

821 Zimmermann, E. (2010). Vocal expression of emotion in a nocturnal prosimian primate  
822 group, mouse lemurs. In: Ed. by Brudzynski, S. M, *Handbook of Mammalian Vocalization:*  
823 *An Integrative Neuroscience Approach*, (pp. 215–226). Oxford: Academic Press.  
824  
825