Ontogeny of long-range vocalizations in a Neotropical fossorial rodent: the Anillaco Tuco-Tuco (*Ctenomys* sp.)

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Adult vocalizations can develop following three alternative modes; by retention of juvenile vocalizations, through the modification of juvenile precursors or by a *de novo* appearance in adults. Vocalizations that develop from juvenile precursors may develop following two pathways; vocal learning (implying the ability of juveniles to modify their vocalizations based on an external auditory input) and/or vocal tract maturation (involving the improvement of the capacity of juveniles to generate progressively more adult-like vocalizations by the tuning of an innate motor program). The emission of adult vocalizations requires the synchronization of neuromuscular and anatomical structures, and the lack of maturation and/or precise coupling between them would lead to the production of abnormal vocalizations. Tuco-tucos (*Ctenomys* spp.) are subterranean rodents that produce territorial, high intensity long-range vocalizations (LRVs) of, broadband and low frequency that are essential for long-distance communication between individuals in different tunnel systems. Despite their importance, the developmental modes, pathways and developmental sequences of LRVs remain poorly understood. In adult Anillaco Tuco-Tucos (*Ctenomys* sp.) the LRV is composed by two types of syllables (series and individual notes) that are repeated a variable number of times. We studied the development of the LRV in 8 juveniles of the Anillaco Tuco-Tuco ranging from 14 to 108 days after birth. The LRV exhibited a mixed developmental mode: while series developed from juvenile precursors whose acoustic features gradually approached those of adults, individual notes appeared later in the ontogeny and *de novo*, with acoustic features undistinguishable from those of adults. Vocalizations became progressively longer, but the series types shown by adults were evident in juveniles at an early age. All three acoustic features of series (triad) notes studied correlated with age in both sexes (duration 90% increased through development, while bandwidth 90% and peak frequency decreased). LRV developed normally in juveniles acoustically isolated from adults, supporting the vocal

tract maturation hypothesis and possibly rejecting the vocal learning hypothesis. Juveniles emmitted a higher proportion (7.4%) of abnormal vocalizations than adults (0.3%), as expected in the development of any complex behavior that requires practice to be mastered. The maturation of the LRV occurred well before the sexual maturation, presumably due to the protracted time needed to acquire or build a burrow system long before mating is possible. We propose that protracted vocal development is another component in the slow developmental strategy of *Ctenomys* and subterranean rodents in general.

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2 **Tuco (*Ctenomys* sp.)**

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13 **Abstract**

14 Adult vocalizations can develop following three alternative modes; by retention of juvenile
15 vocalizations, through the modification of juvenile precursors or by a *de novo* appearance in
16 adults. Vocalizations that develop from juvenile precursors may develop following two
17 pathways; vocal learning (implying the ability of juveniles to modify their vocalizations based on
18 an external auditory input) and/or vocal tract maturation (involving the improvement of the
19 capacity of juveniles to generate progressively more adult-like vocalizations by the tuning of an
20 innate motor program). The emission of adult vocalizations requires the synchronization of
21 neuromuscular and anatomical structures, and the lack of maturation and/or precise coupling
22 between them would lead to the production of abnormal vocalizations. Tuco-tucos (*Ctenomys*
23 spp.) are subterranean rodents that produce territorial, high intensity long-range vocalizations
24 (LRVs) of, broadband and low frequency that are essential for long-distance communication
25 between individuals in different tunnel systems. Despite their importance, the developmental
26 modes, pathways and developmental sequences of LRVs remain poorly understood. In adult
27 Anillaco Tuco-Tucos (*Ctenomys* sp.) the LRV is composed by two types of syllables (series and
28 individual notes) that are repeated a variable number of times. We studied the development of
29 the LRV in 8 juveniles of the Anillaco Tuco-Tuco ranging from 14 to 108 days after birth. The LRV
30 exhibited a mixed developmental mode: while series developed from juvenile precursors whose
31 acoustic features gradually approached those of adults, individual notes appeared later in the
32 ontogeny and *de novo*, with acoustic features undistinguishable from those of adults.
33 Vocalizations became progressively longer, but the series types shown by adults were evident

34 in juveniles at an early age. All three acoustic features of series (triad) notes studied correlated

35 with age in both sexes (duration 90% increased through development, while bandwidth 90%

36 and peak frequency decreased). LRV developed normally in juveniles acoustically isolated from

37 adults, supporting the vocal tract maturation hypothesis and possibly rejecting the vocal

38 learning hypothesis. Juveniles emmitted a higher proportion (7.4%) of abnormal vocalizations

39 than adults (0.3%), as expected in the development of any complex behavior that requires

40 practice to be mastered. The maturation of the LRV occurred well before the sexual maturation,

41 presumably due to the protracted time needed to acquire or build a burrow system long before

42 mating is possible. We propose that protracted vocal development is another component in the

43 slow developmental strategy of *Ctenomys* and subterranean rodents in general.

44

45 **Introduction**

46 Like many other aspects of biological systems, vocalizations experiment an ontogeny. In

47 principle, adult vocalizations could develop following three alternative modes. Adults may just

48 retain the vocalizations of juveniles (Campbell et al. 2014), vocalizations may develop through

49 the modification of juvenile precursors that eventually reach adult features (Grimsley et al.

50 2011), or they may appear *de novo* in adults without the need of homologous juvenile

51 precursors (Campbell et al. 2014). Additionally, vocalizations in the early life generally differ

52 from those in adulthood, either because they perform specific functions that are lost with aging

53 (e.g., begging vocalizations) (Hammerschmidt et al. 2000, 2001; Hauser 1989; Seyfarth &

54 Cheney 1986) or because the rate of emission of the same vocalization changes from being

55 common in juveniles to rare in adults or viceversa (e.g., the trill of *Cebus capucinus*) (Gros-Louis

56 2002).

57 Vocalizations that develop from juvenile precursors into adult ones, may follow two non

58 mutually exclusive developmental pathways: vocal learning and vocal tract maturation (Janik &

59 Slater 1997). Development of vocalizations by vocal learning implies the ability of juveniles to

60 modify their vocalizations based on the copying of sounds from an external auditory input,

61 typically from conspecifics (Tyack 2016). Juveniles of vocal learners can modify the acoustic

62 features of their vocalizations and also incorporate new vocalizations to their repertoire as a

63   result of the influence of social interactions (Janik & Slater 1997). On the other hand,

64   development of vocalizations by vocal tract maturation involves the improvement of the

65   capacity of juveniles to generate progressively more adult-like vocalizations by the tuning of an

66   innate motor program to anatomical structures without the need of an auditory input (Winter

67   et al. 1973; Tyack 2016).

68   The acquisition of adult vocal patterns requires practice, since it is necessary to

69   synchronize the neuromuscular system and anatomical structures, either to match the vocal

70   output to those signals acquired through external input or to achieve the successful

71   deployment of instructions contained in the internal innate motor program (Boughman & Moss

72   2003). The lack of maturation and/or precise coupling between the morphology and the

73   neuromuscular system of the vocal apparatus leads to the production of abnormal

74   vocalizations. Consequently, regardless of the developmental pathway followed, juveniles are

75   expected to produce a greater proportion of abnormal vocalizations during their practice

76   period than adults during the emission of their mature vocalization (Konishi 1965).

77   Tuco-tucos (*Ctenomys* spp.) are subterranean rodents that produce long-range

78   vocalizations (LRVs) of high intensity, broadband and low frequency. These vocalizations are

79   essential for long-distance communication between individuals in different tunnel systems

80   (Schleich & Busch 2002, Amaya et al. 2016). Multiple lines of evidence suggest that LRVs are

81   territorial signals that facilitate the maintenance of individual territories and minimize

82   aggressive encounters, especially between males (Francescoli 1999; Schleich & Busch 2002;

83   Amaya et al. 2016). Despite their behavioral importance, the LRVs of adults of only three of the

84   more than 60 recognized species of *Ctenomys* (Bidau 2015) have been characterized in some

85   detail (Francescoli 1999, Schleich & Busch 2002, Amaya et al. 2016). Juvenile LRVs were simply

86   mentioned in *C. talarum* (Zenuto et al. 2002) and *C. mendocinus* (Camin 2010), but there is no

87   detailed information on the developmental modes, pathways or developmental times of LRVs.

88   Learning how and when the LRVs reach maturity can help understand how life-history traits

89   interact and influence each other in fossorial rodents. For example, since burrows are

90   energetically expensive resources (Vleck 1979, 1981) it is conceivable that solitary species of

91   *Ctenomys* secure or build their own burrow system before mating. This would imply the need of

92   having mature LRVs to help defend the burrows during the process of acquiring a mate, even

93   before reaching sexual maturity.

94   The LRV of adult Anillaco Tuco-Tucos (*Ctenomys* sp.) is composed by two types of

95   syllables (series and individual notes) that are repeated a variable number of times (Figure 1). In

96   normal vocalizations, each series begins with a note that is followed immediately by a soft note,

97   but adults occasionally produce structurally abnormal series by adding a soft note before the

98   initial note (Amaya et al. 2016). Depending on the number of notes sandwiching soft notes,

99   series can be classified as dyads (two notes, a soft note), triads (three notes, two soft notes), or

100 tetrads (four notes, three soft notes) (Amaya et al. 2016). In contrast, individual notes are single

101 notes emitted at a faster rate in succession and are always given after the series (Figure 1).

102   This paper aims to describe the ontogenetic changes of the LRVs in the Anillaco Tuco-

103 Tuco based on recordings of captive-reared pups, in order to 1) discuss which developmental

104 mode (juvenile retention, juvenile precursor, or *de novo* appearance) explains the ontogeny of

105 the two types of syllables of the long-range vocalization of the Anillaco Tuco-Tuco; 2) evaluate

106 whether the ontogeny of the long-range vocalizations of the Anillaco Tuco-Tuco fits the vocal

107 learning and/or the vocal tract maturation developmental pathways; 3) test the prediction that

108 juveniles should produce a greater proportion of anomalies in precursors of the LRV, than

109 adults when giving the fully mature rendering of this vocalization, and 4) discuss the

110 relationship between the development of LRVs and other life-history traits.

111

Materials and methods

Experimental animals

114   We studied the development of the LRV in 8 juveniles (5 males and 3 females) of the

115 Anillaco Tuco-Tuco (see Amaya et al. 2016 for taxonomic background). Juveniles were trapped

116 at Anillaco, La Rioja, Argentina (28°48'50"S, 66°55'54"O; 1365 m asl) in November and

117 December yearly between 2013 and 2016. Immediately after trapping, juveniles were

118 acoustically isolated from adults and placed in individual enclosures in the Chronology and

119 Ethology lab in CRILAR (see Amaya et al. 2016 for enclosure features and animal care data).

120   Juveniles were weighed four times (when trapped and after 30-35 days in captivity; Table 1)

121   and sexed *a posteriori* because the genitalia were not mature at the time of capture.

122   Since we captured weaned juveniles of unknown biological age, we estimated their

123   absolute ages at trapping by comparing their weights to those of juvenile *C. mendocinus* of

124   known age (Camin 2010). Adults of the Anillaco Tuco-Tuco are 10% heavier than adults of *C.*

125   *mendocinus* (Camin 2010, Amaya et al. 2016)*.* Thus, we assumed a linear age-weight

126   relationship such that 10% heavier juveniles of the Anillaco Tuco-Tuco where aged as

127   equivalent to 10% lighter juveniles of *C. mendocinus* (e.g., at day 14 juveniles of *C. mendocinus*

128   weigh on average 33 g, while at this age the weight of juvenile Anillaco Tuco-Tucos would be

129   around 36.3 g). The minimum estimated age at trapping was 14 days (female 1; Table 1) and

130   the maximum 28 days (male 1; Table 1).

131

132   **Recording of vocalizations**

133   We studied the development of LRVs of 8 juveniles of Anillaco Tuco-Tuco. Each juvenile was

134   recorded in four 6-8hr sessions in alternate days within a week every four weeks (i.e., a week of

135   recording with four sessions was separated by three weeks of not recording). Sessions began

136   during the first week that individuals were trapped and finished after twelve weeks. Thus, each

137   individual was recorded in 16 sessions spread along 12 weeks, except for male 3 that died in the

138   ninth week and was recorded in only 12 sessions.

139   Recordings were made from outside the enclosure using a Zoom H4n digital hand

140   recorder system with built-in microphones (sample rate of 44.1 kHz and 24 bit depth) mounted

141   on a tripod with the microphones facing the enclosure. The gain setting of the recorder was the

142   same for all recordings. Our recording protocol allowed us to detect and record the emission of

143   the LRVs, which occur at a low rate and unpredictably. All the recording sessions were made

144   during the night (between 22:00 and 6:00hs Argentina time, GMT -5), due the low level of

145   ambient noise and to the high nocturnal activity of the species in laboratory conditions

146   (Tachinardi et al. 2014). In total, we recorded 5 males for 448 hours in 76 sessions and 3

147   females for 276 hours in 48 sessions.

148   We searched for LRVs in recordings by manually examining on-screen spectrograms

149 built with Raven pro 1.4 (http://www.birds.cornell.edu/Raven). We used the following

150 spectrogram parameters to prioritize resolution in time to be able to easily detect vocalizations:

151 Window: Hann; size: 256 samples (= 5,8 ms); 3 dB bandwith-filter: 248 Hz; Time grid-overlap:

152 50%; hop size: 128 samples (= 2,9 ms); Frequency grid–DFT size: 256 samples; grid spacing: 172

153 Hz.

154

155

156

157 **Acoustic characterization of the long-range vocalization**

158 *Acoustic characterization of series*. We acoustically characterized triads of juveniles because

159 they are the most common and characteristic series type of the LRV in adults of these species

160 (Amaya et al. 2016). To compare triads of juveniles with those of adults we characterized triads

161 in three adult males and three adult females (three triads per adult individual), using adult

162 recordings from Amaya et al. (2016). We measured duration 90% (ms), bandwidth 90% (Hz) and

163 peak frequency (Hz) of each note in one triad per vocalization in juveniles and adults. We

164 calculated duration 90%, bandwidth 90% and peak frequency for each triad note as follows.

165 First, we delimited triads with a selection border A (400 ms and 0-22.05 kHz) (Figure 2 A);

166 second, we divided the selection border A in 20 equal selections borders B (20 ms and 0-22.05

167 kHz) (Figure 2 B); third, we calculated the energy contained within each B selection border

168 (Figure 2 B); and fourth, we defined a selection border C (40 ms and 0-22.05 kHz) for each triad

169 note choosing the two adjacent selection borders B with the highest energy values (Figure 2 B).

170 We calculated single duration 90%, bandwidth 90% and peak frequency values for each

171 individual in each session by averaging the values of the three triad notes.

172

173 **Acoustic characterization of individual notes**. We analyzed all juvenile vocalizations to quantify

174 the number of individual notes emitted per vocalization and acoustically characterized them. To

175 compare individual notes of juveniles with those of adults we characterized individual notes in

176 three adult males and three adult females, using adult recordings from Amaya et al. (2016). We

177   measured duration 90% (ms), bandwidth 90% (Hz) and peak frequency (Hz) in three individual

178   notes per vocalization in juveniles and adults. We calculated duration 90%, bandwidth 90% and

179   peak frequency for each individual note as follows. First, we delimited individual notes with a

180   selection border D (80 ms and 0-22.05 kHz) (Figure 2 B); second, we divided the selection

181   border D in 4 equal selections borders E (20 ms and 0-22.05 kHz) (Figure 2 C); third, we

182   calculated the energy contained within each E selection border (Figure 2 C); and fourth, we

183   defined a selection border F (40 ms and 0-22.05 kHz) for each individual note choosing the two

184   adjacent selection borders E with the highest energy values (Figure 2 C). We calculated single

185   duration 90%, bandwidth 90% and peak frequency values for each individual in each session by

186   averaging the values of the three individual notes.

187

188   **Spectrogram parameters**. All acoustic measurements were made with Raven Pro 1.4. We used

189   different spectrograms parameters that allowed us to obtain accurate time and frequency

190   domain measurements. For duration 90% we used the following spectrogram parameters:

191   Window: Hann; size: 256 samples (= 5,8 ms); 3 dB bandwith-filter: 248 Hz; Time grid-overlap:

192   50%; hop size: 128 samples (= 2,9 ms); Frequency grid–DFT size: 256 samples; grid spacing: 172

193   Hz. For bandwidth 90% and peak frequency the spectrogram parameters were: Window: Hann,

194   size: 1024 samples (= 52.4 ms), 3 dB bandwith-filter: 27.4 Hz; Time grid–overlap: 50%, hop size:

195   512 samples (= 26.2 ms); Frequency grid–DFT size: 4,096 samples, grid spacing: 4.7 Hz. All

196   recordings were band-pass filtered between 80–5,000 Hz in Raven Pro 1.4 to eliminate sources

197   of disturbance and distortion in acoustic measurements as in Amaya et al. (2016).

198

199   **Series types and abnormal series**

200   We analyzed all juvenile vocalizations to quantify the occurrence of each of the three normal

201   series-patterns (dyad, triad and tetrad). We first identified the series-patterns in the

202   corresponding waveform and spectrogram by counting the number of notes and soft-notes per

203   series, and then counted the number of series-patterns per vocalization. Following the same

204   procedure, we quantified the occurrence of abnormal series in juvenile vocalizations. We

205   considered a series to be abnormal if it had a soft-note before the note 1 in dyads, triads and

206 tetrads or if it was composed by a single note followed by a soft-note (i.e., it was a monad)

207 (Figure 3).

208   In order to test the prediction that juveniles produce proportionally more abnormal

209 series than adults, we compared the proportion of abnormal series in adults (Amaya et al.

210 2016) to the proportion of abnormal series in juveniles. To calculate these proportions we

211 counted the number of abnormal series and divided these counts by the total number of series

212 in juveniles and in adults (325 juvenile series recorded for the present study and 703 adult

213 series from Amaya et al. [2016]).

214

215 **Statistical analyses**

216 To evaluate if the acoustic features of juvenile triads notes changed during their development,

217 we performed Spearman correlations between duration 90%, bandwidth 90% and peak

218 frequency (one average value per variable was calculated using the three triad notes for each

219 individual at each sampling week) against absolute age for each individual. To evaluate if the

220 number of series per vocalization changed along the development we performed a Spearman

221 correlation between the number of series per vocalization and absolute age for each individual.

222   To examine if juvenile triads changed more quickly at some point in time or whether

223 developmental changes were even through the sampled period, we averaged acoustic features

224 from all recording sessions within a week for all individuals of the same sex and of similar age

225 (i.e., within an 11 day range in males and a 9 day range in females) and compared these values

226 between all weeks and against adult values of the corresponding sex using a Kruskall-Wallis

227 test. The same procedure was applied to compare the acoustic characteristics of the individual

228 notes of each juvenile male and of the average value of all males of similar age (i.e., within a

229 108-118 day range) against adult values.

230

231   All procedures followed the guidelines of the American Society of Mammologists for the

232 use of wild mammals in research (Sikes & Gannon, 2011). All experiments were performed at

233 the CRILAR in Anillaco and were authorized by the Environmental Department of La Rioja

234 (permits 028–10 and 062–08) and approved by the Ethics Committee of the Faculty of

235 Veterinary Sciences of La Plata National University, Argentina (permit 29-2-12). All individuals

236 were returned to their natural environment after the study was complete. For statistical

237 analyses we used InfoStat (Di Rienzo et al. 2012).

238

239 **Results**

240 Seven juveniles of the Anillaco Tuco-Tuco (five males and two females) produced 71 LRVs in the

241 12 study weeks; while an eighth juvenile (female 3) did not produce any vocalization (Table 1).

242 The two syllable types of LRVs, series and individual notes, appeared sequentially in the vocal

243 development (Figure 4). Series appeared first and were given by juveniles as young as 14 days

244 old (39 g). Individual notes were given considerably later in the development, when juveniles

245 were as young as 108 days old (156 g) (Figure 4, Table 1). Accordingly, most vocalizations were

246 composed only by series (95.77%, N=68), and fewer vocalizations were composed by series and

247 individual notes (4.22%, N=3) (Table 1).

248

249 **Development of series**

250 Developmental changes caused juvenile triads to progressively approach the acoustic

251 parameters of adult triads (Figure 5). The seven juveniles that vocalized gave series (Table 1).

252 The number of series per vocalization increased through development and varied from 1 to 25

253 in juvenile males (Table 1, Table S3) and from 1 to 6 in juvenile females (Table 1, Table S3). The

254 first vocalizations of all juveniles contained a single series, and the highest numbers of series

255 were recorded in juvenile males between 108 to 118 days old, and in juvenile females between

256 104 to 113 days old (Table 1).

257   Juveniles produced the three series-patterns present in the adults. Triads were by far

258 the most common series-pattern (82.76%, N=269), followed by dyads (16.61%, N=54), and

259 tetrads (0.61%, N=2) (Table 1). Triads were produced by all vocalizing juveniles, dyads were

260 produced by all juveniles (except male 3 which died prematurely), and tetrads were produced

261 by only two juveniles (female 1 and female 2) (Table 1).

262   Within individual developmental trajectories, duration 90% exhibited a directional

263 increase through development and correlated with age in two out of four males males and in

264 both females (Table 1, Table S3), whilst bandwidth 90% of triad notes decreased in all but one

265 individual (Table S3), and peak frequency of triad notes decreased during the development of

266 all individuals (Table S3). When individuals were lumped by sex, all three acoustic features of

267 triad notes correlated with age: duration 90% increased through time (males: r= 0.25,

268 P<0.0001; females: r = 0.55, P <0.0001), while bandwidth 90% (males: r= -0.49, P<0.0001;

269 females: r= -0.75, P<0.0001) and peak frequency decreased (males: r= -0.58, P<0.0001; females:

270 r= -0.49, P<0.0001).

271   Acoustic parameters progressively approached features of adults in both sexes and

272 achieved mature features at different developmental stages (Table 2). All acoustic parameters

273 passed through one or two intermediate developmental stages in which parameters were

274 statistically different from adults as they approached adult values (Table 2).

275

Abnormal series in juveniles and adults

277 Juveniles emitted two types of abnormal series during the development of the long-range

278 vocalization. In the first type of abnormal series a soft note preceded the first series note, and

279 included abnormal triads (Figure 3A), dyads (Figure 3B) and tetrads (Figure 3C). In the second

280 type, the series was conformed by a single monad that consists of a single note followed by a

281 soft-note (Figure 3D). Monads are unknown in adults (see Amaya et al. 2016).

282   The proportion of abnormal to normal series was obviously larger in juveniles than in

283 adults. While in juveniles 7.38% of the series were abnormal (8 abnormal triads; 11 abnormal

284 dyads; 3 abnormal tetrads; 4 monad vs 325 normal series), in adults only 0.28 % of the series

285 were abnormal (one abnormal triad and one abnormal dyad vs. 701 normal series).

286

Development of individual notes

288 Only 3 of the 5 males and none of the two females that vocalized gave individual notes. These

289 notes were given by males 1, 2 and 4 when they had between 156-162 g and were 108-118

290 days old (Table 1); while male 3 died at an early age, male 5 approached this weight and was

291 within this age range but never gave individual notes (Table 1). Individual notes were recorded

292 in only a single vocalization in each male, and ranged in number from 22-31 notes always

293   emitted after a sequence of series and never in isolation (Table 1). Acoustic parameters

294   (duration 90%, bandwidth 90% and peak frequency) of individual notes of all juveniles were

295   undistinguishable from adults both individually and when averaged (Kruskal Wallis test, p>0.05)

296   (Table 1, Figure 5).

297

298

299   **Discussion**

300   In this paper we have shown that the LRV of the Anillaco Tuco-Tuco (*Ctenomys* sp.) exhibits a

301   mixed developmental mode in which the two syllables types of the vocalization develop

302   differently: while series developed from juvenile precursors whose features gradually approach

303   those of adults, individual notes appeared later in the ontogeny and *de novo*, with acoustic

304   features undistinguishable from those of adults. We also conclude that the LRV develops

305   normally in juveniles acoustically isolated from adults, supporting the vocal tract maturation

306   hypothesis and possibly rejecting the vocal learning hypothesis. Finally, we found that juveniles

307   emit a higher proportion of abnormal vocalizations than adults, as expected in the development

308   of any complex behavior that requires practice to be mastered.

309

310   **Development of the long-range vocalization**

311   The LRV in the Anillaco Tuco-Tuco exhibits a mixed developmental mode, since it includes a

312   syllable type (series) that develops gradually from structurally conserved ontogenetic

313   precursors in the juveniles and another syllable type (individual notes) that appears *de novo*

314   with adult features. This sort of mixed development is novel and has not been reported in the

315   vocalizations of other rodents. For example, while the vocalization of *Mus musculus* develops

316   from precursors of the syllables that increase in duration and decrease in fundamental

317   frequency (Grimsley et al. 2011), the acoustic characteristics of the notes in *Scotinomys* spp. do

318   not experience ontogenetic changes (Campbell et al. 2014) and are therefore the product of

319   retention of juvenile vocalizations.

320   Triads are by far the most common series pattern in isolated captive juveniles and in

321   free-ranging adults of the Anillaco Tuco-Tuco (Amaya et al. 2016). Early in the ontogeny,

322   juveniles of the Anillaco Tuco-Tuco produce the three series-patterns present in adults (dyads,

323   triads and tetrads), but the early acoustic features of triads differ from those of adults. Juvenile

324   precursor triad notes decrease bandwidth and peak frequency gradually until reaching the

325   values of adult triads. Thereby, juvenile precursors develop into adult series by gradual changes

326   in their acoustic parameters but without changing their basic structure. This lack of changes in

327   the structure of series is interesting since *a priori*, other developmental mechanisms could have

328   occurred. For instance, it would have been possible to begin with the emission of separate

329   notes and soft-notes, passing to their joint emission in the shape of monads, subsequently

330   leading through the repetition of this basic structure to the creation of the other types of series

331   composed by more notes and soft-notes. We interpret the early appearance of triads and their

332   ubiquitous presence as evidence of a substantial genetic makeup in the structure of series in

333   *Ctenomys*. In fact, other series-patterns predominate in other species of tuco-tucos. For

334   example, monads are characteristic of *Ctenomys talarum* (Schleich & Busch 2002) and dyads

335   are characteristic of *Ctenomys mendocinus* (Amaya et al. 2016, Amaya and Areta, unpublished

336   data).

337   The lower degree of morphological and neuromuscular maturation and tunning in the

338   vocal tract of juvenile in comparison to adult tuco-tucos may account for the higher proportion

339   of abnormal series in young individuals. This explanation may be more general and could also

340   apply to juveniles of two species of singing mice (*Scotinomys teguina and S. xerampelinus*). Five

341   types of notes were described in juvenile *Scotinomys* spp. (Campbell et al. 2014); one very

342   frequent note (note A) exists at all ages and is almost the only note sang by adults, while four

343   less frequent notes are more frequent in the first 12 days of life and become almost non-

344   existent after 30 days (Campbell et al. 2014). We speculate that the four types of less frequent

345   notes are not different notes *per se,* but that they could simply represent anomalies of the note

346   A. Hence, their decrease in frequency could be explained by the improved performance of the

347   vocal tract and associated neuromuscular apparatus through development.

348   Individual notes of LRVs appear late, *de novo* and with acoustic parameters

349   indistinguishable from those of adults, showing a developmental mode that is strikingly

350   different to that of series. The first individual notes are given by juveniles when the series have

351 already acquired adult acoustic characteristics through maturation from precursors. This

352 suggests that the appearance of individual notes with their definitive characteristics without

353 the existence of ontogenetic precursors is possible because the neuromuscular system and the

354 vocal apparatus have acquired the necessary "fine tuning" through practice of the series during

355 their development. The passive maturation of the individual notes might be, at least in part, a

356 co-product of the active maturation of the series.

357   The LRVs in the Anillaco Tuco-Tuco exhibit a large variety of syntactic patterns (Amaya et

358 al. 2016). These syntactic patterns undergo some general and predictable ontogenetic changes

359 that include, first an increase in both the number of series and series-patterns per vocalization,

360 and then an increase in syllable types given by the late appearance of individual notes. The

361 increment in song length and the late appearance of individual notes might be widespread in

362 *Ctenomys*. These patterns appear to occur also in the vocal development of *C. mendocinus*,

363 where "short calls" presumably conformed only by series occur at 33 days and "long calls"

364 presumably conformed by series and individual notes occur at 70 days (Camín 2010). In

365 contrast, the highly stereotyped pattern of *Scotinomys* adult song appears *de novo* in the first

366 vocalization of juveniles after a period of vocal inactivity (Campbell et al. 2014).

367

368 **Vocal learning vs vocal tract maturation in the LRV**

369 Vocalizations of rodents appear to develop by maturation of the vocal tract and not by vocal

370 learning since adult vocalizations develop normally without the need of an external auditory

371 model from which to copy (Scherrer & Wilkinson 1993, Grimsley et al. 2011). In this paper we

372 demonstrated that juveniles of the Anillaco Tuco-Tuco develop normal LRVs without an

373 external auditory input between 14 and 108 days. This would indicate that the development of

374 vocalization is a product of vocal tract maturation and not of a vocal learning process (Efremova

375 et al. 2011). However, juveniles were exposed to adult LRVs from birth until they were

376 captured, and *Ctenomys* are born with an open auditory meatus (Zenuto et al. 2002, Camín

377 2010). Thus, it remains possible that adult vocalizations heard at an early age have served as an

378 auditory model for the subsequent vocal development. To thoroughly test the importance of

379 vocal learning in the development of LRVs in *Ctenomys,* future studies should focus on

380 discovering when juveniles acquire the ability to listen and on evaluating if deafened juveniles

381 develop normal LRVs when devoid from acoustic self-stimulation (Kroodsma & Konishi 1991;

382 Campbell et al. 2014).

383

384

385 **Life-history traits and territorial vocalizations in subterranean rodents**

386 Subterranean rodents exhibit slow life-histories, which are characterized by slow speed of

387 tissue growth due to a low basal metabolic rate selected for by the high energetic demands of

388 burrowing (Henneman, 1983, 1984; Hofman, 1983; Martin, 1984a , 1984b; Glazier 1985)

389 leading to delayed sexual maturity (Busch et al. 2000). In *Ctenomys talarum* and *C. mendocinus*

390 gestation lasts around 95 days (Zenuto et al. 2002; Camin 2010); sexual maturity takes 180-240

391 days in *C. talarum* (Busch et al. 2000) and presumably adult vocalizations have been reported

392 long before sexual maturity at 65-70 days (Zenuto et al. 2002) and the same pattern is evident

393 in *C. mendocinus* which reaches sexual maturity around 180-270 days (Camín 2010) and alleged

394 vocal maturity at 70 days (Camín 2010). We lack precise information on the age of sexual

395 maturity in the Anillaco Tuco-Tuco, but the patterns described for *C. talarum* and *C.*

396 *mendocinus* suggest that vocalizations mature well in advance of sexual maturity in *Ctenomys*.

397 The solitary bathyergids, *Bathyergus janetta, B*. *siullius* and *Georinchus capensis* exhibit a

398 moderate gestation period of around 50 days and presumably delayed sexual maturation (Jarvis

399 1969; Bennett & Jarvis 1988; Bennett et al. 1991, 2000). In *B. janetta* and *B. siullius* juveniles

400 begin to produce seismic signals (foot-drumming) at 80 days, after dispersion at 60-65 days

401 from the maternal burrow system, while in *Georinchus capensis* juveniles produce foot-

402 drumming at 50 days of age before dispersion at 55-60 days of age (Bennett & Jarvis 1988).

403 Dispersion in these bathyergids, occurr when they have the capacity to dig and protect their

404 own burrow systems (Bennett & Jarvis 1988). Thus, territorial acoustic signals of solitary

405 ctenomyids and bathyergids seem to mature approximately at the time of dispersion but long

406 before sexual maturity.

407 The protracted development of the LRV of the Anillaco Tuco-Tuco (ca. 110-120 days)

408 herein reported contrasts markedly with the short developmental times of vocalizations of

409 superficial rodents such as *Scotinomys* (35 days; Campbell et al. 2014) and *Mus musculus* (at 13

410 days individuals had some adult vocal characteristics, Grimsley et al. 2011). The altricial

411 *Ctenomys* contrast with the precocial *Scotinomys* in which gestation takes 30 days (Hopper &

412 Carleton 1976) and which reach sexual maturity after 30-40 days concomitantly with the

413 maturation of adult vocalizations (Hopper & Carleton 1976, Campbell et al. 2014).

414 We propose that protracted vocal development is another component in the slow

415 developmental strategy of *Ctenomys* and subterranean rodents in general. The underground

416 burrow systems have a central role in the ecology of subterranean rodents (Busch et al. 2000)

417 and their defense is of key importance. Burrows are costly resources that serve for breeding

418 and that are defended by the use of long-range vocalizations in *Ctenomys* (Amaya et al. 2016).

419 The maturation of vocalizations in advance of sexual maturation might be explained by the

420 protracted time needed to acquire or build a burrow system long before mating is possible.

421

422

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534

Figure 1

General structure and syllable types of the long-range vocalization of the Anillaco Tuco-Tuco (*Ctenomys* sp.).

General structure (above) and syllable types (series and individual notes notes) (below) of the long-range vocalization (LRV) of the Anillaco Tuco-Tuco (*Ctenomys* sp.). Waveforms are shown above and corresponding spectrogram below in both figures. Triad series are composed by three notes (N1, N2 and N3) and two soft-notes (Sa and Sb), and individual notes are simple sounds.

![Spectrogram figure showing acoustic signal analysis. Top panel displays a waveform and spectrogram with Frequency (kHz) on the y-axis. A boxed region is magnified below. The lower panel shows a zoomed view labeled "Series" with segments N1, Sa, N2, Sb, N3, and "Individual notes". Below is a spectrogram with Frequency (kHz) axis marked 0 and 3, and Time (s) axis marked 0.5 and 1.]

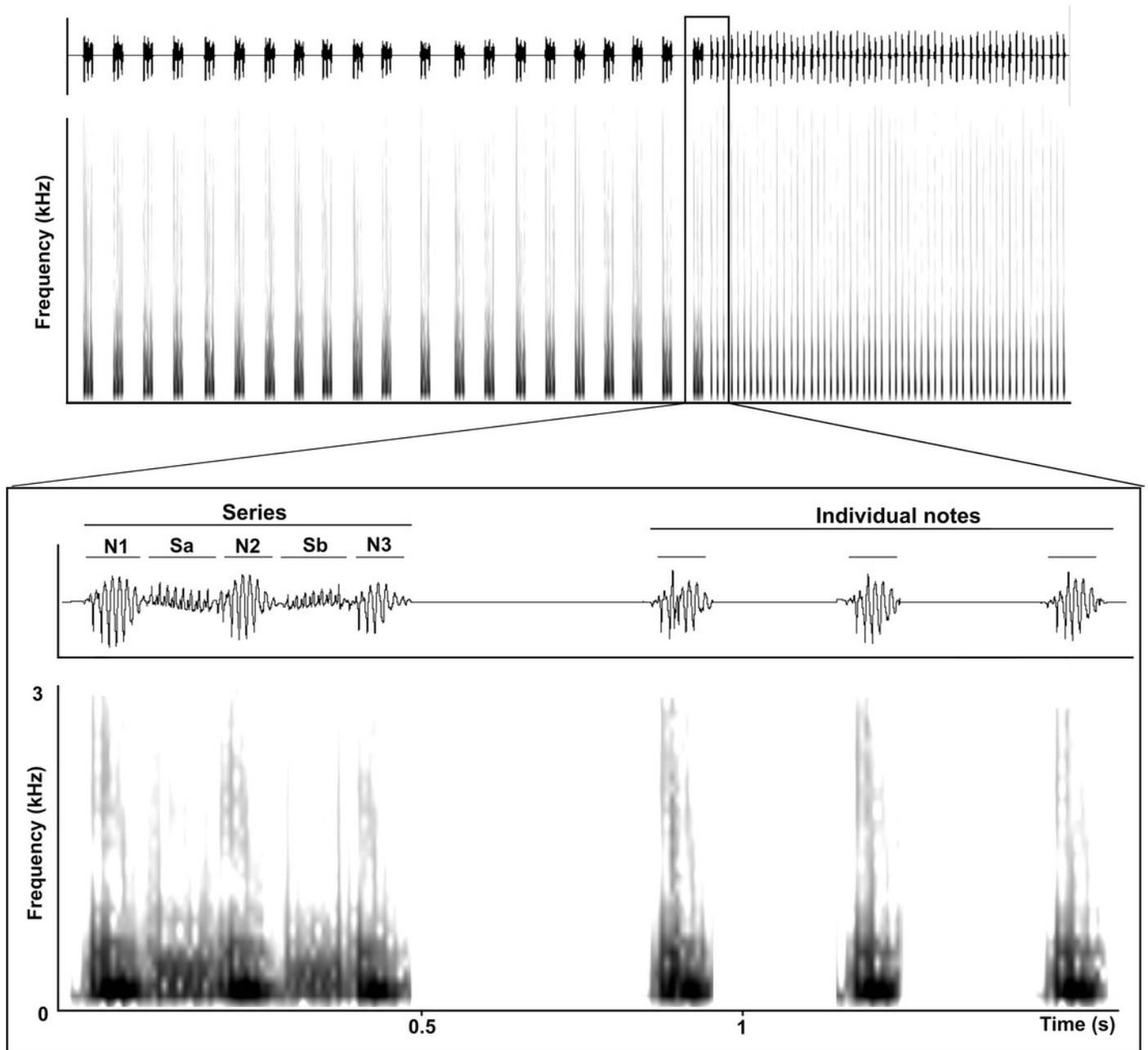



Figure 2

Acoustic characterization of the long-range vocalization during the vocal development of juveniles and in adults of the Anillaco Tuco-Tuco (*Ctenomys* sp.).

Delimitation of selection borders used for the quantitative acoustic characterization of triads (A and B) and individual notes (C and D) of the long-range vocalization (LRV) during the vocal development of juveniles and in adults of the Anillaco Tuco-Tuco (*Ctenomys* sp.). Waveforms are shown above and corresponding spectrogram below in all figures. A. Selection border A delimiting a triad. B. Selection borders B and selection border C delimited by the two adjacent selection borders B with the highest energy values. C. Selection border D delimiting an individual note. D. Selection borders E and selection border F delimited by the two adjacent selection borders B with the highest energy values. We measured duration 90%, bandwidth 90% and peak frequency in selection borders C (in triads) and F (in individual notes).

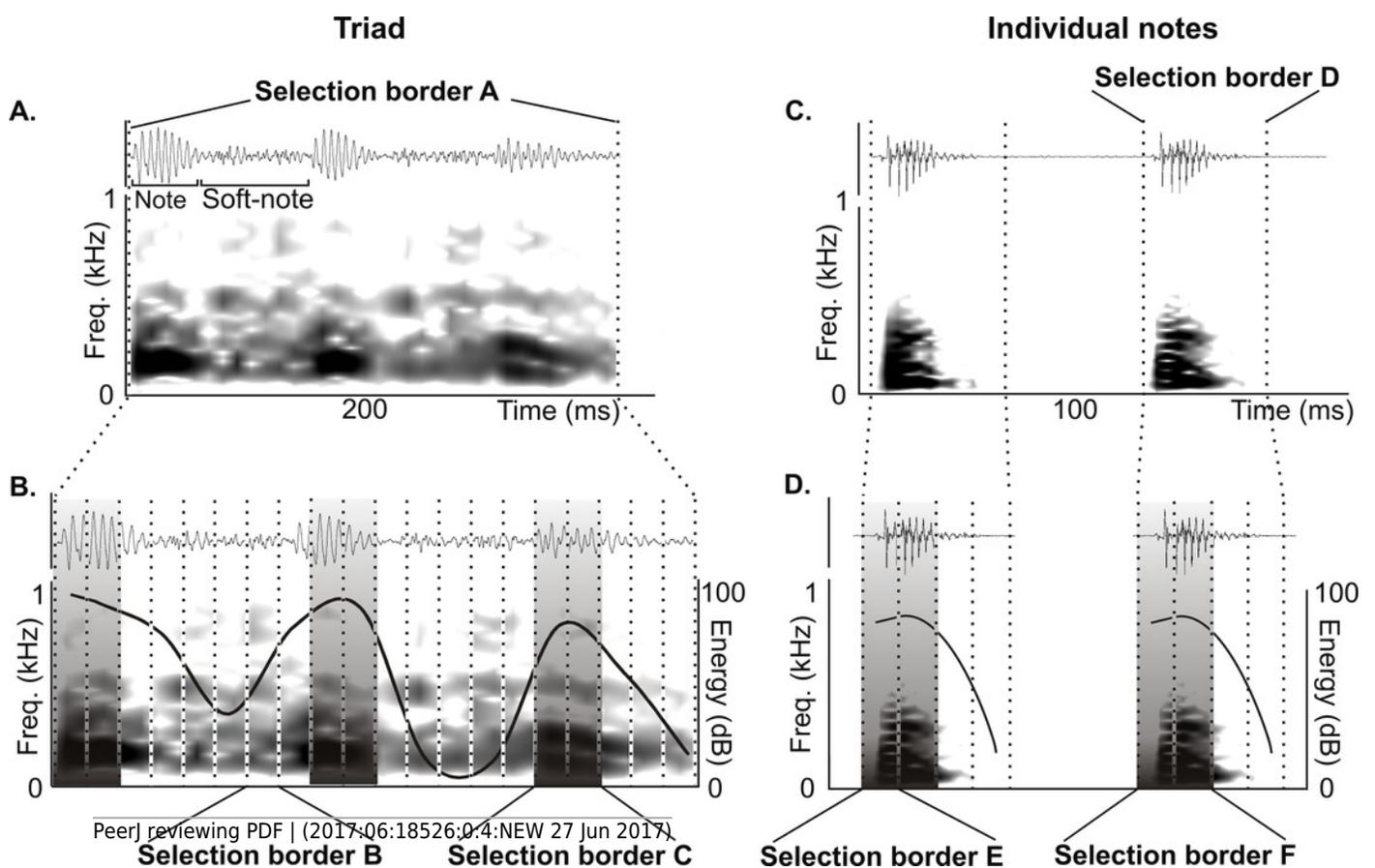



Figure 3

Abnormal series produced by juveniles of the Anillaco Tuco-Tuco (*Ctenomys* sp.)

Abnormal series produced by juveniles of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during the development of the long-range vocalization (LRV). Waveforms are shown above and corresponding spectrogram below in all figures. A. Abnormal triads. B. Abnormal dyads. C. Abnormal tetrad. D. Monad. Previous soft-note (P S); note (N); soft-note (S).

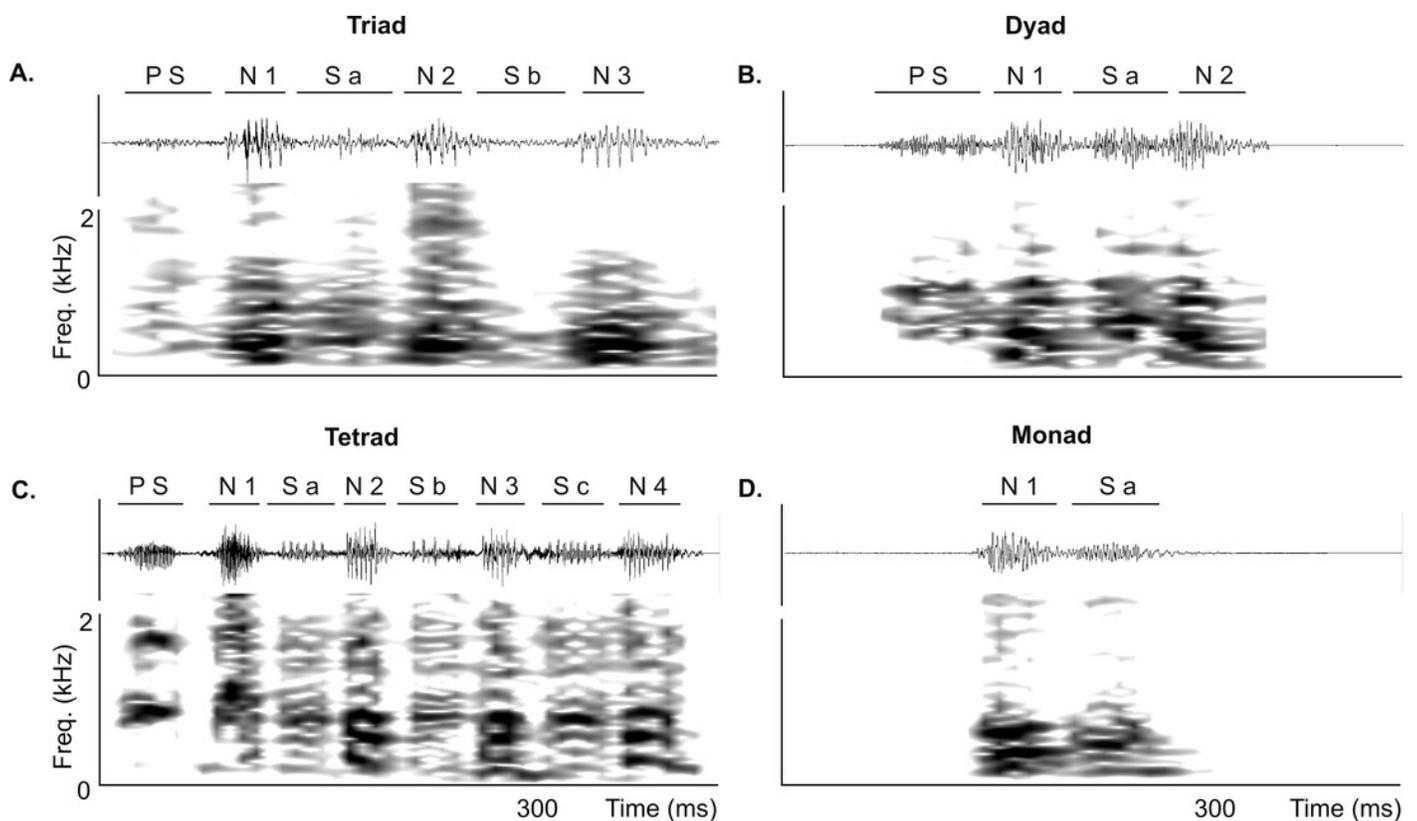



Figure 4

Development of the long-range vocalization

Development of the long-range vocalization (LRV) in a male 2 of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during 4 sampling weeks over 12 study weeks. During the sampling period this individual (male 2) gave 11 vocalizations. Note the increase in the number of series per vocalization and the late appearance of individual notes. These two features are representative of the development of the LRV in this species. For each sampling week we indicate weight (g) and estimated age (days after birth).

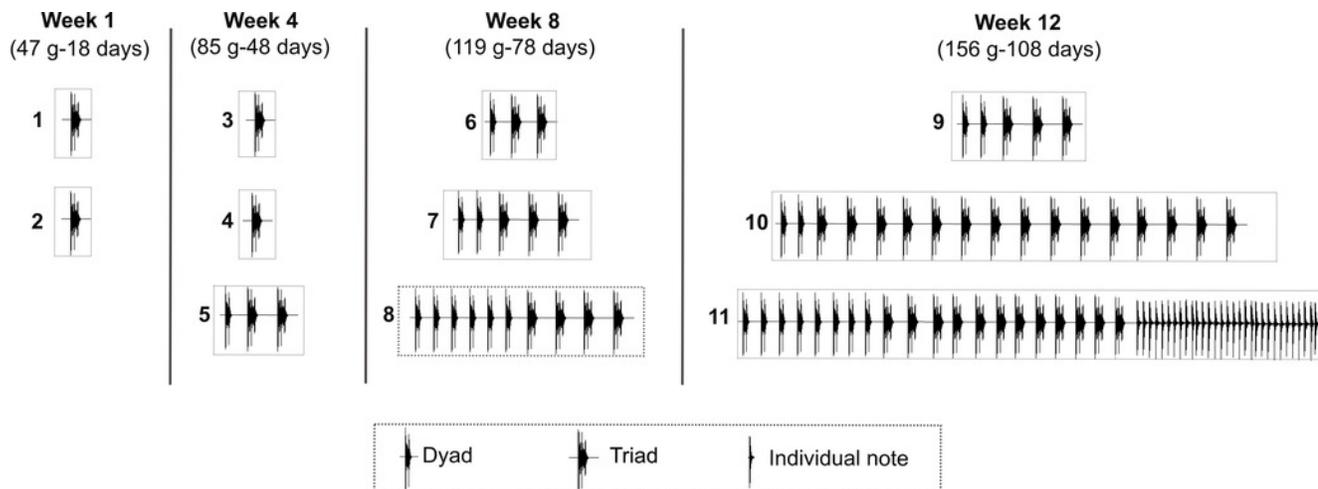
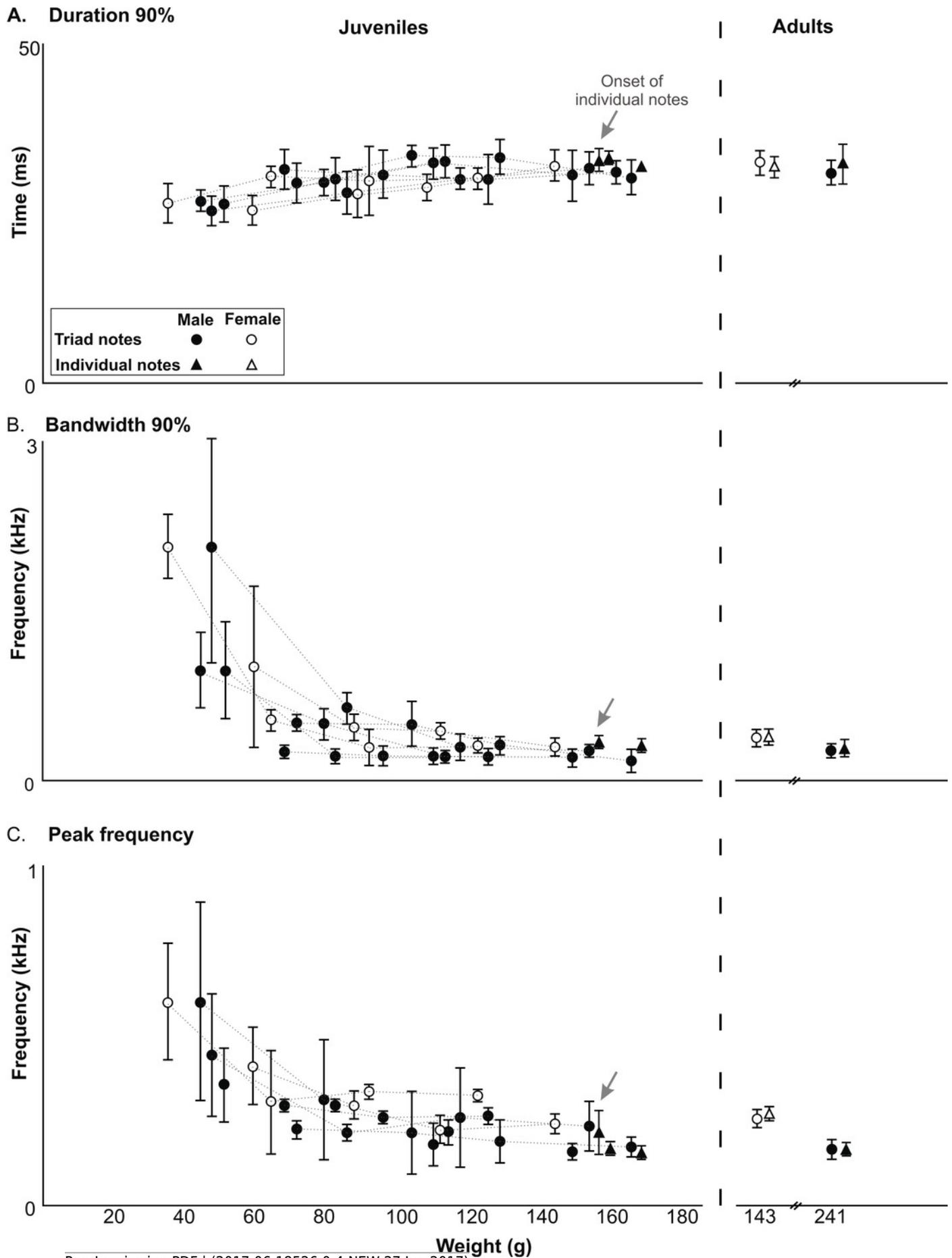



Figure 5

Developmental changes in acoustic features in the long-range vocalization of the Anillaco Tuco-Tucos (*Ctenomys* sp.)

Developmental changes in acoustic features of triad notes and individual notes in the long-range vocalization (LRV) of the Anillaco Tuco-Tucos (*Ctenomys* sp.) and comparison to adult values. All data from juveniles and adults in Table S1 and S2. A. Duration 90%. B. Bandwidth 90%. C. Peak frequency.

Long-range vocalizations emitted by eight juveniles of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during vocal development

Long-range vocalizations emitted by eight juveniles (five males and three females) of the Anillaco Tuco-Tuco (*Ctenomys* sp.) during 4 sampling weeks over 12 study weeks. For each juvenile individual we indicate weight (**W**) (g), estimated age in days (**D**), and recording time in hours (**R**). For each vocalization recorded (Roman numerals) we indicate number of each normal series-pattern (triads in bold font; dyads in normal font; and tetrads in italics), and number of individual notes per vocalization (underlined). Deceased (†), no vocalization (-).

Juveniles individual	Week 1							Week 4							Week 8							Week 12						
	W	D	R	I	II	III	IV	W	D	R	I	II	III	IV	W	D	R	I	II	III	IV	W	D	R	I	II	III	IV
Male 1	73	28	22	1	1	1	-	101	58	24	1	1	1 1	-	125	88	18	2 3	2 3	1 6	-	162	118	24	2 4	10 18 26	2	-
Male 2	47	18	22	1	1	-	-	85	48	20	1	1	1 2	-	119	78	24	1 2	2 3	6 4	-	156	108	24	2 3	2 16	8 12 31	-
Male 3	45	17	20	1	1	-	-	80	47	22	1	2	-	-	117	77	20	4	7	-	-	†	†	†	†	†	†	†
Male 4	71	27	24	1	1	3	-	97	57	24	1 1	3	5	-	123	87	28	2 5	13	2 9	-	158	117	28	13	4 21 22	-	-
Male 5	49	19	24	1	1	-	-	81	49	24	2	4	-	-	111	79	28	1 1	2 5	4 3	-	150	109	28	5	1 6	15	-
Female 1	39	14	22	1	1	*1*	-	68	44	24	1	1	1 1	-	95	74	22	1	2	3	-	125	104	20	5	6	-	-
Female 2	59	23	24	1	2	*1*	-	85	53	24	1	1 1	-	-	110	83	28	1	3	-	-	142	113	28	2	3	6	-
Female 3	55	21	20	-	-	-	-	70	51	18	-	-	-	-	92	81	22	-	-	-	-	115	101	24	-	-	-	-

Pairwise comparisons of acoustics parameters in series notes of the long-range vocalization of the Anillaco Tuco-Tuco (*Ctenomys* sp.).

Pairwise comparisons (Kruskal-Wallis test) of duration 90%, bandwidth 90% and peak frequency of series notes at different developmental segments of the long-range vocalization (LRV) of the Anillaco Tuco-Tuco (*Ctenomys* sp.). Shared letters indicate no statistical differences, different letters indicate significant statistical differences between developmental segments.

	Males				
Acoustic	**Juveniles (days of age)**				**Adults**
parameter	**17-28**	**47-58**	**77-88**	**108-118**	
Duration 90%	A	B	B	B	B
Bandwidth 90%	A	A	B	B	B
Peak frequency	A	B	B	C	C
	Females				
Acoustic	**Juveniles (days of age)**				**Adults**
parameter	**14-23**	**44-53**	**74-83**	**104-113**	
Duration 90%	A	B	B	B C	C
Bandwidth 90%	A	A	A	B	B
Peak frequency	A	B	BC	BC	C