1 **Evolutionary adaptations for the temporal processing of natural sounds by the anuran**

2 **peripheral auditory system**

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9 Running title: Temporal processing in treefrogs

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21 **Keywords** Auditory evoked potentials • Green treefrog • Temporal Processing • Communication •

22 Grey treefrog • Hearing

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Abstract

Sensory systems function most efficiently when processing natural stimuli, such as vocalizations, and it is thought that this reflects evolutionary adaptation. Among the best-described examples of evolutionary adaptation in the auditory system are the frequent matches between spectral tuning in both the peripheral and central auditory systems of anurans (frogs and toads) and the frequency spectra of conspecific calls. Tuning to the temporal properties of conspecific calls is less well established, and in anurans has so far been documented only in the central auditory system. Using auditory evoked potentials, we asked whether there are species-specific or sex-specific adaptations of the auditory systems of gray treefrogs (*Hyla chrysoscelis*) and green treefrogs (*H. cinerea*) to the temporal modulations present in conspecific calls. Modulation rate transfer functions (MRTFs) constructed from auditory stead-state responses (ASSRs) revealed that each species was more sensitive than the other to the modulation rates typical of conspecific advertisement calls. In addition, auditory brainstem responses (ABRs) to paired-clicks indicated relatively better temporal resolution in green treefrogs, which could represent an adaptation to the faster modulation rates present in this species' calls. MRTFs and recovery of ABRs to paired-clicks were generally similar between the sexes, and we found no evidence that males were more sensitive than females to the temporal modulation patterns characteristic of the aggressive calls used in male-male competition. Together, our results suggest that efficient processing of the temporal properties of behaviorally relevant sounds begins at potentially very early stages of the anuran auditory system that include the periphery.

Introduction

A prominent hypothesis in systems neuroscience is that sensory systems are most efficient when processing natural stimuli (Atick, 1992; Barlow, 1961; Hateren, 1992; Laughlin, 1981; Simoncelli and Olshausen, 2001). This efficiency reduces energy and resource expenditure associated with sensory processing. Auditory systems appear well adapted to process the spectral and temporal features of natural sounds, such as speech and other communication signals (Rieke et al., 1995; Singh and Theunissen, 2003; Smith and Lewicki, 2006; Suga, 1989; Woolley et al., 2005). Often such adaptations manifest as selectivity for behaviorally relevant sounds, which helps increase detectability of signals relative to background noise (Machens et al., 2005; Rieke et al., 1995). For example, the spectro-temporal tuning of neurons in the midbrain and forebrain of songbirds facilitates discrimination between conspecific songs, while limiting interference from modulations inherent in less-behaviorally relevant sounds (Woolley et al., 2005).

Research on anuran amphibians (frogs and toads) yielded some of the first examples of auditory adaptations to natural sounds (Capranica and Moffat, 1975; Frishkopf et al., 1968; Mudry et al., 1977; Narins and Capranica, 1976). In most anuran species, males have repertoires of calls that are used for mate attraction and resource defense. In the periphery, one or both of the two inner ear sensory papillae for detecting airborne sound – the amphibian papilla (AP) and the basilar papilla (BP) – and their afferents are predominantly tuned to the acoustic frequencies emphasized in conspecific calls (Capranica and Moffat, 1983; Frishkopf et al., 1968; Narins and Capranica, 1980; Ryan et al., 1992). Neurons in the central auditory system are also predominantly tuned to acoustic frequencies in conspecific calls, with some combination-sensitive neurons firing only when multiple frequencies from conspecific calls are present (Fuzessery and Feng, 1982; Fuzessery and Feng, 1983; Hall, 1994; Megela, 1983; Mudry and Capranica, 1987a; Mudry and Capranica, 1987b; Mudry et al., 1977). This matched spectral filtering (Capranica and Moffat, 1983; Simmons, 2013) by both the peripheral and central nervous systems represents an evolutionary adaptation that facilitates coding of the frequency spectra of vocalizations, which are especially important natural stimuli for frogs.

In addition to spectral properties, temporal properties of anuran calls are also critical for species and call recognition and intraspecific discrimination (Castellano and Rosso, 2006; Gerhardt, 1978; Gerhardt and Doherty, 1988; Rose and Brenowitz, 2002; Schwartz, 1987; Walkowiak and Brzoska, 1982). There is evidence for the operation of matched temporal filters in the central auditory system, but less so in the periphery (Rose and Gooler, 2007; Simmons, 2013). In the central auditory system, neurons exhibit preferences for specific temporal properties of calls such as the rate of pulses or amplitude modulation (AM) (Diekamp and Gerhardt, 1995; Eggermont, 1990; Gooler and Feng, 1992; Walkowiak, 1984), inter-pulse interval (Alder and Rose, 1998; Edwards et al., 2002), and duration (Condon et al., 1991; Gooler and Feng, 1992; Narins and Capranica, 1980; Penna et al., 1997) using rate codes. In the case of AM, the distributions of AM rates preferred by neurons in the central auditory system are often centered near the pulse rates or modulation rates characteristic of

82   conspecific calls, suggesting specialization for the neural encoding of the temporal patterns present in

83   conspecific signals (Diekamp and Gerhardt, 1995; Penna et al., 2001; Rose and Capranica, 1984;

84   Rose and Capranica, 1985; Rose et al., 1985). In contrast to the rate code common in central auditory

85   neurons, auditory nerve fibers encode temporal properties in the timing of their impulses. For

86   example, nerve fibers use a periodicity code to encode AM by phase-locking, or discharging at a

87   particular phase of the modulation cycle (Dunia and Narins, 1989; Feng et al., 1991; Rose and

88   Capranica, 1985). The ability of auditory nerve fibers to phase-lock to AM tends to decrease as a

89   function of increasing modulation rate (Dunia and Narins, 1989; Feng et al., 1991; Rose and

90   Capranica, 1985). While several studies have verified the ability of anuran auditory nerve fibers to

91   phase-lock to temporal modulations in the amplitude envelopes of conspecific signals (Capranica and

92   Moffat, 1975; Frishkopf et al., 1968; Klump et al., 2004; Schwartz and Simmons, 1990; Simmons et

93   al., 1992; Simmons et al., 1993), there is so far little evidence for enhanced peripheral selectivity

94   favoring the temporal modulations typical of conspecific calls.

95   The broad aim of this comparative study was to investigate features of temporal processing by

96   the peripheral auditory system that might reflect adaptations for encoding temporal modulations

97   present in conspecific vocalizations. We conducted our experiments using two well-studied frogs,

98   Cope's gray treefrog (*Hyla chrysoscelis*) and the green treefrog (*H. cinerea*) (Bee, 2012; Bee, 2014;

99   Gerhardt, 1982; Gerhardt, 2001; Gerhardt and Huber, 2002). The advertisement calls that males of

100   each species produce differ in both spectral and temporal properties (see Fig. 1). The advertisement

101   call of gray treefrogs is comprised of a series of short (e.g., 10 ms), temporally discrete pulses

102   delivered at species-specific rates of about 40 to 65 pulses/s (Ward et al., 2013). Pulses have energy at

103   frequencies of about 1.25 kHz and 2.5 kHz, with the lower frequency peak attenuated by about 11 dB

104   relative to the higher peak (Ward et al., 2013). In contrast, the advertisement call of the green treefrog

105   consists of a single, biphasic note (120-200 ms; Gerhardt, 1974a) having an initial pulsed phase, with

106   pulse rates ranging between about 100 to 200 pulses/s, followed by an un-pulsed phase having marked

107   waveform periodicity of about 300 Hz (typically ranging from about 200 to 400 Hz) (Oldham and

108   Gerhardt, 1975). These calls contain spectral peaks of approximately equivalent amplitude, with one

109   near 0.9 kHz and a second, broader peak between about 2.5 and 3.6 kHz (Gerhardt, 1974a).

110   In addition to advertisement calls, males of both species also use aggressive calls in disputes

111   with other males over possession of calling sites. The aggressive calls of gray treefrogs exhibit AM in

112   the range of 50 to 100 Hz, though they typically lack the distinct pulsatile structure of advertisement

113   calls (MS Reichert, personal communication, June 2014; Reichert and Gerhardt, 2014). The

114   aggressive calls of green treefrogs are similar to their advertisement calls, but are pulsed throughout at

115   rates near 50 pulses/s (ranging between 39 and 56 pulses/s (Oldham and Gerhardt, 1975). Female

116   treefrogs strongly prefer advertisement calls to aggressive calls (Brenowitz and Rose, 1999; Marshall

117   et al., 2003; Oldham and Gerhardt, 1975; Schwartz, 1986; Schwartz, 1987; Wells and Bard, 1987).

118   Given their importance in male-male competition for calling sites, aggressive calls are likely more

119   behaviorally salient to males than females.

120   We investigated temporal processing using auditory evoked potentials (AEPs). AEPs measure

121   neural activity from the auditory nerve and brainstem in response to acoustic stimuli, and they are a

122   common tool for studying auditory processing in humans and other animals (Brittan-Powell et al.,

123   2010a; Brittan-Powell et al., 2010b; Gall et al., 2013; Hall, 2007; Henry and Lucas, 2008; Higgs et al.,

124   2002; Katbamna et al., 1992; Kenyon et al., 1998; Ladich and Fay, 2013; Popov and Supin, 1990;

125   Supin et al., 1993). We used two well-established AEP techniques that have been used previously to

126   investigate temporal processing, the auditory steady state response (ASSR) evoked by AM tones and

127   the auditory brainstem response (ABR) evoked by paired acoustic clicks (Burkard and Deegan, 1984;

128   Dolphin and Mountain, 1992; Gall et al., 2013; Henry and Lucas, 2008; Mann et al., 2005; Purcell et

129   al., 2004; Wysocki and Ladich, 2005). The magnitude of the ASSR reflects the degree of neural

130   synchronization to AM in the signal, and thus the ASSR measures the ability of the auditory system to

131   track temporal fluctuations in amplitude (Dolphin and Mountain, 1992; Gall et al., 2012; Mann et al.,

132   2005). ASSR magnitude can be plotted as a function of AM to generate modulation rate transfer

133   functions (MTRFs) (Fig. 2). MRTFs typically have an overall low-pass shape consistent with phase-

134   locking in the auditory nerve (Dolphin and Mountain, 1992; Dolphin et al., 1994; Dolphin et al., 1995;

135   Finneran et al., 2007; Gall et al., 2012). In the present study, we recorded ASSRs in response to tones

136   of three different carrier frequencies modulated at AM rates between 12.5 Hz and 800 Hz (in 1-octave

137   steps). For each species, the specific carrier frequencies (denoted low, middle, or high, in reference to

138   their relative frequencies) were selected based on the species-specific tuning of the AP and BP in our

139   two study species. We recorded ABRs in response to paired clicks, in which the time between the

140   clicks (inter-click interval, ICI), varied between trials (Fig. 3). This double-click procedure measures

141   the ability of the auditory system to resolve two sounds in close temporal proximity (Burkard and

142   Deegan, 1984; Henry et al., 2011; Supin and Popov, 1995a; Wysocki and Ladich, 2002). We focused

143   these analyses on the first peak of the ABR (P1; Fig. 4), which is thought to be generated by the

144   auditory nerve (Achor and Starr, 1980; Buchwald and Huang, 1975; Seaman, 1991). Performance was

145   measured in terms of percent recovery, which we calculated as the amplitude (as in Fig. 4) of the

146   response to the second click in a pair as a percentage of the amplitude of the response to a single-

147   click. Additionally, we calculated the minimum resolvable ICI at which a response to the second click

148   could be detected.

149   We used data from ASSR and ABR recordings to test two hypotheses related to the temporal

150   processing of natural sounds. The *species-specific adaptation hypothesis* holds that the auditory

151   system is specialized to process temporal features characteristic of conspecific advertisement calls

152   compared with those more typical of heterospecific calls. We based this hypothesis on species-

153   differences between advertisement calls for two reasons. Advertisement calls are by far the most

154   common vocalization produced by males of both species. These signals are used in both mate

155 attraction and call site defense and, thus, are behaviorally relevant to both sexes (Garton and Brandon,

156 1975; Ritke and Semlitsch, 1991; Wells, 1977). Our comparative approach allowed us to make the

157 following prediction: gray treefrogs should have relatively larger ASSRs than green treefrogs at the

158 relatively slower modulation rates (e.g. between 25 and 100 Hz) near the pulse rates of gray treefrog

159 advertisement calls, while green treefrogs should have relatively larger ASSRs than gray treefrogs to

160 stimuli with relatively faster modulation rates close to those typical of the faster modulations present

161 in green treefrog advertisement calls (e.g. between 100 and 400 Hz). These species differences should

162 be reflected in a species × modulation rate interaction in analyses of MRTFs. We also predicted that,

163 in ABRs evoked by paired clicks, green treefrogs would show faster recovery of responses to the

164 second click and shorter minimum resolvable ICIs than gray treefrogs, because tracking the faster

165 modulation rates in the green treefrog advertisement call should require greater temporal resolution.

166 The *sex-specific adaptation hypothesis* holds that males should exhibit greater selectivity than

167 females for the temporal features of conspecific aggressive calls. This hypothesis follows from the

168 inference that aggressive calls, which are used in male-male interactions, are more behaviorally

169 salient to males than females. According to this hypothesis, we predicted that a species × sex ×

170 modulation rate interaction would influence the shape of MRTFs. In gray treefrogs, the temporal

171 modulations present in aggressive calls (50 to 100 pulses/s) are slightly faster than those in

172 advertisement calls (40 to 65 pulses/s); therefore, we predicted MRTFs for male gray treefrogs would

173 be skewed toward faster modulation rates than those of conspecific females (i.e. relatively larger

174 ASSRs between 50 and 100 Hz in males). In response to paired clicks, we also predicted male gray

175 treefrogs, compared with conspecific females, would have faster ABR recovery and shorter minimum

176 resolvable ICIs. In green treefrogs, aggressive calls exhibit temporal modulations at rates between 39

177 and 56 pulses/s; therefore, we predicted male green treefrogs would have greater ASSRs than

178 conspecific females at modulation rates near 50 Hz. Given that the modulations in advertisement calls

179 of green treefrogs are actually faster than those in male-male aggressive calls, we predicted either no

180 sex difference or relatively faster ABR recovery and shorter minimum resolvable ICIs in females than

181 males in this species.

182

183 **Results**

184 *Species-specific adaptation hypothesis*

185 As in many other animals, the MRTFs for both treefrog species decreased as modulation rate

186 increased (Fig. 5). The effect of modulation rate was significant, and it also had a large effect size

187 compared to the other effects (Table 1). There was no significant main effect of species; however, the

188 species × modulation rate interaction was significant (Table 1). The effects of this interaction can be

189 seen in that each species had larger ASSRs than the other at modulation rates typical of conspecific

190 calls, a result consistent with our predictions. For example, at modulation rates of 25 to 100 Hz, gray

191 treefrogs had significantly larger responses than green treefrogs when stimuli had the highest carrier

192   frequency (Table 2; Fig. 5). In contrast, green treefrogs had larger ASSRs than gray treefrogs at

193   higher modulation rates (e.g. 200 and 400 Hz) for most carrier frequencies (Fig. 5). The difference

194   was significant for responses to modulation rates of 200 Hz at all carrier frequencies (Table 2).

195   Recovery increased as a function of increasing ICI (Table 3), and these functions were overall

196   very similar in shape between the two species (Fig. 6a). There was no significant effect of species on

197   recovery, nor were there significant effects of any of the interactions involving species (Table 3). This

198   result was inconsistent with our predictions. On average, however, green treefrogs were able to

199   resolve slightly shorter ICIs than gray treefrogs ($F_{1,61} = 5.7$, $p = 0.020$, partial $\eta^2 = 0.09$), a result that

200   was consistent with our prediction. The average minimum resolvable ICI was ($\overline{X} \pm$ s.e.m.) 1.6 ± 0.1

201   ms for green treefrogs and 2.0 ± 0.1 ms for gray treefrogs.

202

203   *Sex-specific adaptation hypothesis*

204   Overall, MRTFs were similar between the sexes in both gray treefrogs (Fig. 7a) and green treefrogs

205   (Fig. 7b). In contrast to our predictions, the species × sex × modulation rate interaction was not

206   significant (Table 1). Hence, there was no evidence of larger responses in male gray treefrogs than

207   female gray treefrogs at modulation rates between 50 and 100 Hz, nor did male green treefrogs have

208   larger responses than conspecific females at modulation rates of 50 Hz. There was, however, a

209   significant sex × modulation rate × carrier frequency interaction (Table 1). In response to the middle

210   carrier frequency, females of both species consistently had larger ASSRs than males, a difference that

211   reached significance in response to stimuli with modulation rates between 50 and 400 Hz (Table 2;

212   Fig. 7). Responses to stimuli at the middle carrier frequency overall tended to be larger for females

213   and smaller for males than corresponding responses to stimuli with the low or high carrier frequency.

214   Inconsistent with our predictions, recovery functions differed little between the two sexes

215   (Fig. 6b). Subject sex did not have a significant effect on percent recovery, and the interaction of sex

216   with ICI was also not significant (Table 3). There was no sex-difference in minimum resolvable ICI

217   ($F_{1,61} = 0.5$, $p = 0.469$, partial $\eta^2 = 0.01$), nor was there an interaction between species and sex ($F_{1,61} =$

218   0.2, $p = 0.666$, partial $\eta^2 < 0.01$).

219

220   **Discussion**

221   Our results provide robust support for the species-specific adaptation hypothesis, and no support for

222   the sex-specific adaptation hypothesis. The key to uncovering evidence supporting the species-

223   specific adaptation hypothesis was our comparisons of two species with calls having quite different

224   temporal structures (see Fig. 1). Cope's gray treefrogs have pulsatile advertisement calls, with pulse

225   rates ranging between 40 and 65 pulses/s, whereas the advertisement calls of green treefrogs exhibit

226   temporal modulation at higher rates between 100 pulses/s and 400 cycles/s. While the aggressive calls

227   of gray treefrogs are modulated at faster rates than their advertisement calls, these modulations are

228   slower than the fastest rates in the advertisement and aggressive calls of green treefrogs. At low

229   modulation rates (e.g. near 50 Hz), gray treefrogs tended to have relatively larger ASSRs than green

230   treefrogs, especially in response to stimuli with the highest carrier frequency. In contrast to gray

231   treefrogs, green treefrogs tended to have relatively larger ASSRs at higher modulation rates (e.g. 200

232   Hz), a result consistent across carrier frequencies. Green treefrogs also had relatively shorter

233   minimum resolvable ICIs compared with gray treefrogs. Together, these results based on ASSRs and

234   ABRs suggest gray treefrogs are adapted to process the relatively slower modulation rates found in

235   their calls, whereas green treefrogs are adapted to tracking the relatively faster pulse rates and

236   periodicities in their calls.

237   Species differences in ASSR amplitudes depended on carrier frequency in gray treefrogs, but

238   not green treefrogs, as indicated by the species × modulation rate × carrier frequency interaction. This

239   finding is noteworthy, as it suggests specializations in temporal processing related to differences in

240   how the spectral properties of the two species' vocalizations are transduced. Recall that in both

241   species, each of the two spectral peaks in the advertisement call is primarily transduced by one of the

242   two inner ear sensory papillae (the AP or the BP) (Buerkle et al., 2014; Capranica and Moffat, 1983;

243   Gerhardt, 1974c; Hillery, 1984; Schrode et al., 2014). In gray treefrog advertisement calls, the relative

244   amplitude of the high spectral peak (2.5 kHz) is approximately 11 dB greater than the low spectral

245   peak (1.25 kHz; Ward et al., 2013). Hence, the majority of the acoustic energy in gray treefrog calls

246   falls in the frequency range of the BP. This is relevant because gray treefrogs were better than green

247   treefrogs at processing the *slower* modulation rates typical of gray treefrog advertisement calls only at

248   the high carrier frequencies transduced primarily by the BP. In contrast to gray treefrogs, the low and

249   high spectral peaks of green treefrog calls have comparable relative amplitudes; therefore, both the

250   AP and the BP transduce prominent spectral peaks in green treefrog calls. Green treefrogs were

251   relatively better than gray treefrogs at processing the *faster* modulation rates typical of green treefrog

252   advertisement calls at both the low and the high carrier frequencies transduced primarily by the AP

253   and BP, respectively. Our data, therefore, suggest that species-specific adaptations in temporal

254   processing may be closely tied to potential species-differences in the roles of the two sensory papillae

255   in processing spectral information in conspecific vocalizations. At present, the specific mechanism

256   underlying the species × modulation rate × carrier frequency interaction remains unknown and should

257   be investigated further in future studies.

258   The species-specific adaptations identified in the present study are consistent with the idea

259   that sensory systems are specially adapted to process the temporal patterns of common or behaviorally

260   important natural stimuli. Adaptation of sensory systems to salient stimuli can improve efficiency and

261   accuracy of neural processing (Atick, 1992; Barlow, 1961; Hateren, 1992; Laughlin, 1981; Simoncelli

262   and Olshausen, 2001). In the case of treefrogs communicating acoustically in cacophonous breeding

263   choruses, adaptation of the auditory systems to process the temporal patterns of present in conspecific

264   calls could improve the neural encoding of these signals, while reducing masking and acoustic

265   interference by the calls of syntopically breeding heterospecifics. These improvements could facilitate

266 detection of and discrimination between conspecific calls, impacting both mate choice decisions by

267 females and disputes between males over calling sites.

268   Species-specific adaptations of temporal processing in the auditory system have been recently

269 identified in songbirds, another group of vocal animals. The responses of neurons in the auditory

270 midbrains of zebra finches (*Taeniopygia guttata*) were found to synchronize to the temporal

271 envelopes of SAM noise across a range of modulation rates that closely match the modulations in

272 conspecific songs (Woolley and Casseday, 2005). The tuning of cells in the midbrain to the temporal

273 properties of sounds is also context-dependent. Temporal tuning tended to be sharper in response to

274 conspecific song stimuli compared to behaviorally neutral noise stimuli, even when the modulations

275 contained in the stimuli were similar (Woolley et al., 2006). Woolley et al. (2005) identified a

276 mismatch between the best temporal tuning of neurons in both the auditory midbrain and forebrain

277 areas and the typical modulation rates in conspecific songs. When considering the average response of

278 neurons in the auditory midbrain and also forebrain areas, the strength of temporal tuning increased as

279 a function of modulation rate, while the power of the modulations occurring in conspecific song

280 decreased as a function of modulation rate (Woolley et al., 2005). This pattern of tuning has the effect

281 of attenuating common modulations and amplifying modulations that vary between conspecific songs,

282 potentially increasing the discriminability of songs (Woolley et al., 2005).

283   An important implication of the results of the present study is that species-specific adaptations

284 in temporal processing might occur as early as the auditory periphery in frogs. In both gray treefrogs

285 and green treefrogs, MRTFs based on ASSR magnitudes were nearly log-linear with respect to

286 modulation rate, with responses decreasing as a function of increasing modulation rate. The ASSR is a

287 measure of neural synchronization, with a strong component originating in auditory nerve fibers

288 (Henry and Lucas, 2008; Supin and Popov, 1995b). The shapes of MRTFs in this study are consistent

289 with previous studies of auditory nerve fibers in frogs, which have also reported decreasing neural

290 synchronization as a function of increasing modulation rate (Dunia and Narins, 1989; Feng et al.,

291 1991; Rose and Capranica, 1985). Our data on ABRs in response to double-click stimuli also support

292 the idea that species differences in temporal processing might arise as early as the auditory periphery.

293 The primary generator of the first peak (P1) in the ABRs of all animals studied to date is the auditory

294 nerve (Achor and Starr, 1980; Brown-Borg et al., 1987; Buchwald and Huang, 1975; Lev and

295 Sohmer, 1972; Seaman, 1991). The timing of P1 of the ABR in both gray and green treefrogs

296 corresponds well to the expected latencies of anuran auditory nerve fibers (Buerkle et al., 2014;

297 Schrode et al., 2014). In support of this view, the minimum resolvable ICIs of between 1.5 and 2.0 ms

298 measured in the present study are comparable to the average gap detection times of between 1.2 and

299 2.2 ms reported previously for anuran auditory nerve fibers (Feng et al., 1994). Our results, therefore,

300 support the hypothesis that the well-known adaptations of the frog peripheral auditory system for

301 processing natural sounds in the spectral domain may also extend to processing in the temporal

302 domain.

9

303   It is potentially surprising that species-specific adaptations in temporal processing might arise

304   in the periphery, because the peripheral auditory system is generally considered to function as a

305   lowpass envelope filter (Carney, 1993; Dau et al., 1996; Dolphin et al., 1995; Frisina, 2001).

306   However, adaptations of the peripheral auditory system for particular modulation rates have been

307   identified in at least one previous study. In a comparison of three species of songbirds, Henry and

308   Lucas (2008) found that the two species whose vocalizations contained the fastest modulations also

309   exhibited larger ASSRs than the third species, particularly at rates faster than 950 Hz. These results

310   suggested coevolution of temporal resolution and temporal modulations in conspecific vocalizations.

311   We believe the current study presents the first evidence in favor of species-specific adaptations for

312   processing temporal patterns in conspecific signals at the level of the auditory periphery in anurans.

313   Future comparative work using electrophysiological recordings from single auditory nerve fibers in

314   both gray treefrogs and green treefrogs will be required to confirm this hypothesis.

315   Our demonstration of species differences in temporal processing also sheds important light on

316   potential auditory mechanisms related to the so-called "cocktail party problem" (McDermott 2009). In

317   both humans and frogs, the background noise levels characteristic of large social aggregations

318   fluctuate in amplitude. Human listeners can take advantage of brief "dips" in noise levels to catch

319   acoustic glimpses of target signals of interest (Bacon et al., 1998; Cooke, 2006; Füllgrabe et al., 2006;

320   Vestergaard et al., 2011). This ability is known as "dip listening," and it is thought to be dependent on

321   having auditory temporal resolution sufficient to resolve the fluctuations in the background noise

322   (Festen, 1993; Qin and Oxenham, 2003). Recent comparative psychophysical studies of gray and

323   green treefrogs uncovered a species difference in their abilities to recognize conspecific advertisement

324   calls in the presence of temporally-fluctuating noise (Vélez and Bee, 2010, 2011, 2013; Vélez et al.,

325   2012). Gray treefrogs, but not green treefrogs, were able to listen in dips to achieve a release from

326   auditory masking by chorus-like noises that fluctuated in amplitude over time. Based on this

327   behavioral difference between the two species, we would have expected gray treefrogs to have better

328   temporal resolution than green treefrogs. However, our results do not support this conclusion and

329   possibly suggest precisely the opposite pattern. A relatively larger ASSR indicates greater synchrony

330   of neural responses, an important component of temporal resolution. Across the modulation rates

331   tested, for each species there where instances when it had larger ASSRs than the other species.

332   However, several factors suggest that green treefrogs have better temporal resolution than gray

333   treefrogs. Green treefrogs had larger ASSRs than gray treefrogs in response to far more stimulus

334   conditions, and furthermore, they tended to have larger ASSRs at faster modulation rates, indicating

335   an ability to synchronize to and resolve faster modulation rates. Green treefrogs also had shorter

336   minimum resolvable ICIs based on ABRs than gray treefrogs. Thus, the species-differences in

337   temporal processing that might exist at the level of the auditory periphery reported in the present

338   study appear poorly suited to explain the differences in dip listening abilities previously described for

10

339 these two species. The present study, therefore, highlights the potentially important role of central
340 auditory processes in solving cocktail-party-like communication problems.

341   A final important result from this study is that it failed to uncover evidence for sex differences
342 in temporal processing. We saw no evidence that males had relatively larger ASSRs than females at
343 modulation rates typical of conspecific aggressive calls, nor was there evidence for a sex-difference in
344 percent recovery functions based on ABRs. Instead, we observed frequency-dependent sex differences
345 in which females tended to have relatively larger ASSRs than males at the middle carrier frequency.
346 Previous behavioral studies (Gerhardt, 2005) and recordings of AEPs (Buerkle et al., 2014; Schrode et
347 al., 2014) in treefrogs indicate that sound frequencies between the two spectral peaks of advertisement
348 calls, and correspondingly between the two frequency regions of greatest auditory sensitivity, are able
349 to simultaneously stimulate both auditory papillae in the anuran inner ear. The observation of larger
350 ASSRs in females suggests better recruitment of nerve fibers across the two papillae in females than
351 males. This result is consistent with previous results from recordings of AEPs in these species
352 (Buerkle et al., 2014; Schrode et al., 2014). In those studies, the amplitudes of P1 of tone-evoked
353 ABRs were larger in females than males when tones had intermediate frequencies (1.5 to 2.0 kHz). At
354 present it remains unclear whether this frequency-dependent sex-difference in responses is indicative
355 of an evolutionary adaptation related to some aspect of spectral or temporal processing.
356

357 **Materials and methods**
358 *Subjects*
359 Subjects were 68 gray treefrogs (35 female) and 59 green treefrogs (30 female). Gray treefrogs were
360 collected from Carver Park Reserve (Carver County, MN, USA), Crow-Hassan Park Reserve
361 (Hennepin County, MN, USA), or Lake Maria State Park (Wright County, MN). Green treefrogs were
362 collected from the East Texas Conservation Center (Jasper County, TX, USA). All frogs were
363 collected in amplexus during their respective breeding seasons in either 2011 or 2012. Female gray
364 treefrogs (X̄ ± s.d.: mass = 5.2 ± 1.0 g; SVL = 39.3 ± 2.7 mm) tended to be larger than male gray
365 treefrogs (4.2 ± 0.8 g; 35.8 ± 1.9 mm). In green treefrogs, females (7.4 ± 1.5 g; 49.4 ± 3.0 mm) and
366 males (7.2 ± 1.4 g; 48.0 ± 3.2 mm) were similar in size. After collection, frogs were transported to the
367 laboratory, where they were housed in terraria on a 12-hour light/dark cycle at ambient room
368 temperature (20 ± 2°C). We supplied frogs with fresh water and a regular diet of vitamin-dusted
369 crickets. We tested each subject within three weeks of collection. All animals were collected with
370 permission from the Minnesota Department of Natural Resources (permit #s 17892 & 19061) and
371 Texas Parks and Wildlife (permit # SPR-0410-054) and treated according to protocols approved by
372 the Institutional Animal Care and Use Committee of the University of Minnesota (#1103A97192).
373

374 *General procedures*

375   Equipment and procedures for recording AEPs have been described previously (Buerkle et al., 2014;

376   Schrode et al., 2014). Briefly, we generated all digital stimuli (50 kHz sampling rate, 16-bit) in TDT

377   SigGenRP software (Tucker Davis Technologies, Alachua, FL, USA). TDT BioSigRP software

378   coordinated stimulus output and neural recording through TDT System 3 hardware. Stimuli were

379   broadcast through an Orb Mod 1 speaker (Orb Audio, New York, NY, USA), which was driven by a

380   Crown XLS 202 amplifier (Crown Audio, Inc., Elkhart, IN, USA).

381   Recordings were made inside a MAC-3 radio-shielded mini-acoustical chamber (W × D × H:

382   81.3 cm × 61 cm × 61 cm; Industrial Acoustics Company, Bronx, NY, USA). For recordings, we first

383   immobilized subjects with an intra-muscular injection of d-tubocurarine chloride (3-12 µg/g body

384   weight). Subjects were loosely wrapped in a thin piece of moistened gauze to facilitate cutaneous

385   respiration and seated in a natural position on an acoustically transparent platform, facing the speaker.

386   Temperature was monitored via a Miller & Weber quick-reading thermometer placed against the

387   subject's body wall and ranged between 18.0 and 20.0 °C across recording sessions. We have

388   observed gray treefrogs in amplexus at temperatures between 14 and 23°C and green treefrogs in

389   amplexus at temperatures between 17 and 26°C. We placed subjects so that the rostral edges of their

390   tympana were 30 cm from the face of the speaker. We applied a topical anesthetic (2.5% lidocaine

391   HCl) to the scalp of the subject prior to inserting the tips of three subcutaneous electrodes (1-5 kΩ)

392   under the skin. The recording electrode was located between the eyes and the ground and inverting

393   electrodes were placed adjacent to the two tympana. Neural signals were sampled at a rate of 25 kHz,

394   digitized, and amplified before being transmitted via optic fiber cable to a TDT RZ5 processor and

395   stored for offline analysis. On the rare occasion that a recording was contaminated with an obvious

396   artifact (e.g. due to infrequent buccal pumping motion), that recording was repeated.

397

398   *Auditory steady-state responses (ASSRs) to modulated tones*

399   We generated AM tones by multiplying two sinusoids, one serving as the modulator (100%

400   modulation depth), and the second serving as the carrier signal. Tones were modulated in 1-octave

401   steps at rates of 12.5, 25, 50, 100, 200, 400, and 800 Hz and were of a sufficient duration to ensure

402   that subjects heard at least 10 modulation cycles at each modulation rate. Tones with modulation rates

403   of 12.5 Hz had a duration of 800 ms. All other tones had durations of 400 ms. We used three different

404   carrier frequencies for each species (1.25, 1.625, and 2.5 kHz for gray treefrogs; 0.9, 1.6, and 2.7 kHz

405   for green treefrogs). The low and high carrier frequencies for each species corresponded to

406   frequencies prominent in conspecific advertisement calls (Gerhardt, 1974a; Gerhardt, 1974b; Schrode

407   et al., 2012), and both species tend to be most sensitive to these two frequencies (Buerkle et al., 2014;

408   Hillery, 1984; Lombard and Straughan, 1974; Miranda and Wilczynski, 2009; Penna et al., 1992;

409   Schrode et al., 2014). The middle carrier frequency for each species was chosen because it

410   simultaneously excites the AP and BP at high signal levels (Buerkle et al., 2014; Gerhardt, 2005;

411   Schrode et al., 2014). In Fig. 2 we show six cycles of example stimuli used to elicit ASSRs from

412   green treefrogs.

413   Calibration of signal level was a two-step process. We first calibrated 1-s (unmodulated) tones

414   with frequencies matching the carrier frequencies of the AM tones to 70 dB SPL (re 20 µPa, C-

415   weighted, fast RMS), using the microphone of a Larson Davis System 824 sound level meter (Larson

416   Davis, Depew, NY, USA) placed at the approximate location of the frog's head and facing the

417   speaker. We then matched the peak-to-peak amplitudes of each AM tone to that of the calibrated,

418   unmodulated tone of corresponding frequency. The frequency response of the speaker was flat (± 1

419   dB) across the range of frequencies tested.

420   We recorded two ASSRs to each stimulus from 30 gray treefrogs (15 females) and 30 green

421   treefrogs (15 females); examples from a green treefrog are shown in Fig. 2. Each ASSR consisted of

422   the average of the responses to 400 presentations of the stimulus. We randomized carrier frequencies

423   and modulation rates of 25 – 800 Hz for each subject. Because of their long duration, tones modulated

424   at a rate of 12.5 Hz were presented in a separate block prior to or following tones modulated at other

425   rates. The timing of the 12.5-Hz block (either before or after the other recordings) and the carrier

426   frequency of tones within the block, were randomized for each subject. Recordings of responses to

427   stimuli were notch-filtered at 60 Hz (roll-off: 18 dB/octave) to reduce electrical noise and low-pass

428   filtered at 3 kHz (roll-off: 6 dB/octave). The notch filter was wide enough to attenuate the amplitudes

429   of recorded responses to stimuli with modulation rates of 50 Hz, but it did so equally for both species

430   and both sexes.

431   In our statistical tests of the species-specific and sex-specific adaptation hypotheses, we only

432   considered evoked responses to occur when the peak of the frequency spectrum of the response at the

433   modulation rate of the stimulus was significantly higher than background noise. We accomplished this

434   as follows. First, we determined the frequency spectrum of each ASSR (Fig. 2) by averaging over the

435   two replicate responses to a given stimulus and then performing an FFT analysis (8192 point) over the

436   first 400 ms of the response. The duration of this analysis window was chosen to achieve a frequency

437   resolution suitable for the modulation rates tested, and it ensured inclusion of an integer number of

438   cycles of the modulation stimulus, which is important for avoiding errors in the calculated frequency

439   spectrum (Herdman and Stapells, 2001; John and Picton, 2000; Nachtigall et al., 2007; Supin and

440   Popov, 1995b). Next, we computed an F ratio comparing the power at the modulation rate of the

441   stimulus (e.g., 100 Hz in Fig. 2) to the average power in the 16 FFT bins adjacent to the modulation

442   rate of the stimulus (Cone-Wesson et al., 2002; Dobie and Wilson, 1996; Gorga et al., 2004; Hall,

443   2007; Herdman and Stapells, 2001; Korczak et al., 2012; Picton et al., 2003; Purcell et al., 2004;

444   Valdes et al., 1997; van der Reijden et al., 2005). Bins were approximately 3 Hz in width, so the

445   background noise was estimated for a range of about 48 Hz surrounding the modulation rate of the

446   stimulus. An evoked ASSR was considered to have occurred if the corresponding F ratio exceeded the

447   critical value of $F_{2,32}$ at $\alpha = 0.05$ (where the degrees of freedom in the numerator and denominator are

13

448   2 times the number of frequency bins used to estimate the signal and noise magnitudes, respectively).

449   We repeated this analysis using time windows that included a fixed number of cycles of each

450   amplitude modulation (10 cycles), rather than fixed time windows (in ms). The same pattern of results

451   noted in the text was present in the results following this alternative analysis method, so we present

452   only the results of the analysis with a fixed time window.

453   We investigated the effects of species, sex, modulation rate, and carrier frequency on evoked

454   ASSRs using a linear mixed model in R (R Development Core Team, 2014), which we fitted using the

455   lme4 (Bates et al., 2014) and afex (Singmann, 2014) packages. Our model included species, sex,

456   modulation rate, and carrier frequency as fixed factors, all two-way interactions, and the three-way

457   interactions of modulation rate × carrier frequency with both species and sex. We performed Tukey

458   post-hoc contrasts using the lsmeans package (Lenth, 2014) to compare between levels of different

459   factors in the model. A significance criterion of $\alpha = 0.05$ was used for all analyses.

460

461   *Auditory brainstem responses (ABRs) to paired-clicks*

462   Click stimuli (0.1-ms duration) output through our setup had a broadband spectrum, with a center

463   frequency of approximately 1.6 kHz and 6-dB down points of approximately 0.345 and 2.8 kHz.

464   Paired-clicks consisted of two acoustic clicks, separated by a specified ICI. Examples are illustrated in

465   Fig. 3a. We tested ICIs of 0.5 ms, 0.75 ms, and 1 to 10 ms, in 1-ms steps, with presentation order

466   randomized between subjects. Each presentation of a paired-click stimulus was followed by at least 40

467   ms of silence and then a single-click stimulus. We recorded two replicate ABRs to the paired-click

468   and single-click stimuli, with each replicate consisting of the average response to 1200 presentations

469   of the stimulus. There was a silent interval of at least 40 ms between the single click and the onset of

470   the next stimulus presentation. Click polarity was constant for all three clicks within a presentation,

471   but alternated between each presentation to reduce the microphonic potential. Clicks were calibrated

472   to 80 dB by matching the peak-to-peak amplitude of each click to that of a calibrated 1-s tone with a

473   frequency of 1000 Hz.

474   We recorded ABRs to paired-clicks from 38 gray treefrogs (20 females) and 29 green

475   treefrogs (15 females) (see Fig. 3b). At relatively long ICIs (e.g. 8 ms), separate ABRs to each of the

476   clicks in the paired-click stimuli were usually evident (Fig. 3b,c). However, at shorter ICIs, the ABRs

477   evoked by the first and second clicks overlapped in time. To disambiguate these overlapping ABRs,

478   we derived the response to the second click by aligning the responses to the single and paired clicks at

479   stimulus onset and then subtracting, point-by-point, the first 25 ms of the average response to the

480   single-click from the average response to the paired-click. This subtraction effectively removed any

481   ABR evoked by the first click of the pair, leaving only the residual ABR evoked by the second click

482   (Fig. 3c). Using a custom-written, cursor-based program in Matlab, we measured the amplitude of all

483   residual ABRs and ABRs evoked by single clicks as the peak-to-peak amplitude from the top of the

484   first peak (P1) to the bottom of the subsequent trough (see Fig. 4) (Buerkle et al., 2014; Schrode et

485 al., 2014). If a peak was not visible, we considered the amplitude to be 0 µV. These values were used

486 to calculate the percent recovery as the ratio of the amplitude of a residual ABR to the amplitude of

487 the ABR evoked by the corresponding single-click. For each subject, we also measured the shortest

488 resolvable ICI. After plotting residual ABRs as a function of ICI (as in Fig. 3c), we selected the

489 minimum resolvable ICI as the shortest ICI for which an evoked residual ABR was visually

490 detectable. We used a repeated-measures ANOVA to investigate the effects of species and sex on

491 percent recovery. We tested for significant differences in minimum resolvable ICI using a two-way

492 ANOVA. Species and subject sex were included as fixed factors. We used a significance criterion of α

493 = 0.05 for both analyses and report p-values corrected based on the Greenhouse-Geisser method

494 (Greenhouse and Geisser, 1959) where applicable.

495

503

504 **Author contributions** KMS and MAB conceived of and designed the experiments, KMS performed

505 the experiments, and KMS and MAB wrote the paper.

506

507

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856

857 **FIGURE CAPTIONS**

858 Fig. 1 **Examples of communication calls of gray treefrogs and green treefrogs.** Waveforms of

859 advertisement call are shown for (A) gray treefrogs and (B) green treefrogs. Depicted in (C) and (D)

860 are waveforms of aggressive calls for gray treefrogs and green treefrogs, respectively. Scale bar in (D)

861 applies to all panels. Aggressive call in (C) courtesy of MS Reichert.

862

863 Fig. 2 **Examples of amplitude modulated stimuli and auditory steady state responses (ASSRs).**

864 ASSRs (alternatively known as envelope following responses or amplitude modulation following

865 responses) were recorded in response to AM tones of three different carrier frequencies for each of the

866 two species. The example stimuli shown (top panels) were used with green treefrogs and had a

867 modulation frequency of 100 Hz and carrier frequencies of (A) 0.9 kHz, (B) 1.6 kHz, or (C) 2.7 kHz.

868 Examples of neural responses from a green treefrog to each stimulus are plotted separately in the time

869 (left panels) and frequency (right panels) domains. During recording, signals were notch-filtered at 60

870 Hz and low-pass filtered at 3 kHz (roll-off: 6 dB/octave). For the purposes of this figure, responses in

871 the time domain have been high-pass filtered (cutoff: 25 Hz, 48 dB/octave) to reveal the periodicity in

872 the trace. Note the peak in the frequency spectrum of the response that matches the 100 Hz

873 modulation rate of the stimulus. The magnitude of this peak is indicative of how well the auditory

874 system followed the AM fluctuation in the envelope of the tone and was used to generate the

875 modulation rate transfer functions (MRTFs) depicted in Figs 5 and 7.

876

877 Fig. 3 **Examples of paired-click stimuli and auditory brainstem responses (ABRs).** Subjects were

878 tested with pairs of acoustic clicks that varied in inter-click interval (ICI). (A) Shown here are

879 examples of paired-click stimuli with ICIs ranging between 0.25 ms and 8 ms. (B) Plotted here are

880 examples of ABRs from a green treefrog to the stimuli in (A). (C) To disambiguate responses to

881 paired-clicks with short ICIs, residual ABRs in responses to the second click of each click pair in (A)

882 were derived by point-to-point subtraction of the ABR elicited by a single click (not shown) from that

883 elicited by paired-clicks in (B).

884

885 Fig. 4 **Amplitude of the ABR response to double clicks.** The amplitude of each residual ABR (see

886 Fig. 3) to a paired-click stimulus and each response to a single click were measured as the peak-to-

887 peak amplitude from the top of P1 (x) to the subsequent trough (-). The example shown here is the

888 residual ABR to a paired-click with an ICI of 8 ms. Arrows indicate times of the first and second click

889 presentations.

890

891 Fig. 5 **Modulation rate transfer functions (MRTFs) for each species.** An ASSR was measured

892 from each recording as the magnitude of the peak in the frequency domain at the frequency equivalent

893   to the modulation rate of the stimulus. Responses are plotted separately for each species across the

894   three carrier frequencies tested. Inequalities depict the predicted direction of responses for the species-

895   specific adaptation hypothesis; the shaded regions indicate the range over which differences were

896   predicted, with overlap in the ranges indicated by diagonal striping. The "low," "middle," and "high"

897   carrier frequencies were, respectively, 1.25 kHz, 1.625 kHz, and 2.5 kHz for gray treefrogs and 0.9

898   kHz, 1.6 kHz, and 2.7 kHz for green treefrogs. All error bars represent the s.e.m.

899

900   Fig. 6 **Paired-click recovery functions.** Recovery was calculated as the ratio of the peak-to-peak

901   amplitude (as calculated in Fig. 4) of the residual ABR to the peak-to-peak amplitude of the ABR

902   elicited by a single click. Recovery is plotted separately for (A) each species and (B) each sex, as a

903   function of ICI. All error bars represent s.e.m.

904

905   Fig. 7 **Modulation rate transfer functions (MRTFs) for each sex.** ASSRs are plotted as functions of

906   stimulus modulation rate separately for each sex for (A) gray treefrogs and (B) green treefrogs.

907   MTRFs are shown separately for the low, middle, and high carrier frequencies (see Fig. 5).

908   Inequalities depict the predicted direction of responses based on the sex-specific adaptation

909   hypothesis; the shaded regions indicate the range over which differences were predicted. Error bars

910   represent the s.e.m.

911

912

913 Table 1 Results of the linear mixed model used to assess effects of species and subject sex on
914 ASSRs. Bold indicates significant terms.

term	df	*F*	*p* value	effect size (partial $\eta2$)
intercept	**1,62**	**3910.6**	**<0.001**	**0.98**
species	1,62	0.2	0.629	0.00
sex	**1,62**	**5.5**	**0.022**	**0.08**
modulation rate	**6,768**	**215.6**	**<0.001**	**0.63**
carrier frequency	2,766	0.2	0.811	0.00
species × modulation rate	**6,768**	**18.4**	**<0.001**	**0.13**
species × carrier frequency	**2,767**	**9.3**	**<0.001**	**0.02**
sex × modulation rate	6,768	1.4	0.198	0.01
sex × carrier frequency	**2,766**	**19.0**	**<0.001**	**0.05**
species × sex	1,61	0.7	0.421	0.01
modulation rate × carrier frequency	**12,764**	**6.3**	**<0.001**	**0.09**
species × modulation rate × carrier frequency	**12,764**	**2.6**	**0.003**	**0.04**
sex × modulation rate × carrier frequency	**12,764**	**2.7**	**0.001**	**0.04**
species × sex × modulation rate	6,768	1.3	0.243	0.01
species × sex × carrier frequency	2,765	2.1	0.127	0.01

915

916 Table 2 Results of Tukey posthoc contrasts. Bold indicates significant terms. Abbreviations:
917 Hch, *H. chrysoscelis*; Hcin, *H. cinerea*; F, female; M, male.

contrast	carrier frequency	modulation rate	Δ	estimate	SE	df	*t*	*p* value	effect size (Cohen's *d*)
species	low	**12.5 Hz**	**Hch > Hcin**	**5.3**	**2.4**	**764.2**	**2.2**	**0.026**	**0.16**
		25 Hz		3.1	1.7	561.7	1.8	0.070	0.15
		50 Hz		-0.2	1.6	473.4	-0.1	0.892	0.01
		100 Hz		1.3	1.4	353.1	1.0	0.337	0.10
		200 Hz	**Hcin > Hch**	**-5.5**	**1.5**	**406.5**	**-3.7**	**<0.001**	**0.37**
		400 Hz		-2.7	1.4	373.0	-1.9	0.061	0.19
		800 Hz		1.0	1.6	504.0	0.6	0.533	0.06
	middle	12.5 Hz		3.4	2.4	765.8	1.4	0.153	0.10
		25 Hz	**Hch > Hcin**	**3.7**	**1.7**	**523.8**	**2.2**	**0.027**	**0.19**
		50 Hz		1.4	1.7	529.1	0.9	0.386	0.08
		100 Hz		-0.4	1.5	412.0	-0.3	0.798	0.03
		200 Hz	**Hcin > Hch**	**-5.6**	**1.5**	**412.0**	**-3.8**	**0.000**	**0.37**
		400 Hz		-2.3	1.4	359.1	-1.6	0.103	0.17
		800 Hz	**Hcin > Hch**	**-9.3**	**1.8**	**609.9**	**-5.1**	**<0.001**	**0.41**
	high	12.5 Hz		3.3	2.1	701.3	1.6	0.111	0.12
		25 Hz	**Hch > Hcin**	**5.2**	**1.6**	**493.3**	**3.2**	**0.001**	**0.29**
		50 Hz	**Hch > Hcin**	**5.9**	**1.6**	**467.1**	**3.8**	**<0.001**	**0.35**
		100 Hz	**Hch > Hcin**	**6.2**	**1.4**	**340.1**	**4.5**	**<0.001**	**0.48**
		200 Hz	**Hcin > Hch**	**-3.4**	**1.4**	**352.3**	**-2.4**	**0.016**	**0.26**
		400 Hz		0.7	1.4	340.1	0.5	0.598	0.06
		800 Hz		-1.7	2.8	796.8	-0.6	0.550	0.04
sex	low	12.5 Hz		0.0	2.4	764.2	0.0	0.989	0.00
		25 Hz		-0.2	1.7	561.7	-0.1	0.916	0.01
		50 Hz		-0.3	1.6	473.4	-0.2	0.837	0.02
		100 Hz		0.2	1.4	353.1	0.2	0.869	0.02
		200 Hz		0.7	1.5	406.5	0.5	0.647	0.05
		400 Hz	**F > M**	**5.3**	**1.4**	**373.0**	**3.7**	**<0.001**	**0.39**
		800 Hz		2.3	1.6	504.0	1.4	0.159	0.13
	middle	12.5 Hz		2.6	2.4	765.8	1.1	0.272	0.08
		25 Hz		2.2	1.7	523.8	1.3	0.186	0.12
		50 Hz	**F > M**	**4.3**	**1.7**	**529.1**	**2.6**	**0.011**	**0.22**
		100 Hz	**F > M**	**8.6**	**1.5**	**412.0**	**5.8**	**<0.001**	**0.57**
		200 Hz	**F > M**	**7.5**	**1.5**	**412.0**	**5.0**	**<0.001**	**0.50**
		400 Hz	**F > M**	**6.4**	**1.4**	**359.1**	**4.5**	**<0.001**	**0.48**
		800 Hz		2.5	1.8	609.9	1.4	0.169	0.11
	high	12.5 Hz		0.1	2.1	701.3	0.0	0.965	0.00
		25 Hz		0.4	1.6	493.3	0.2	0.825	0.02
		50 Hz		-0.2	1.6	467.1	-0.1	0.898	0.01
		100 Hz		-1.4	1.4	340.1	-1.0	0.326	0.11
		200 Hz		0.2	1.4	352.3	0.1	0.883	0.02
		400 Hz		-1.8	1.4	340.1	-1.3	0.194	0.14
		800 Hz		2.9	2.8	796.8	1.1	0.294	0.07

918

919 Table 3 Results of the ANOVA used to assess effects of species and subject sex on responses
920 to paired clicks. Bold indicates significant terms.

term	df	F	p value	effect size (partial η^2)
intercept	**1,60**	**1109.8**	**< 0.001**	**0.95**
ICI	**9,540**	**46.1**	**< 0.001**	**0.43**
species	1,60	0.0	0.985	0.00
sex	1,60	0.8	0.377	0.01
species × ICI	9,540	1.3	0.267	0.02
species × sex	1,60	1.3	0.264	0.02
sex × ICI	9,540	0.8	0.490	0.01
species × sex × ICI	9,540	1.7	0.170	0.03

921

A gray treefrog

B green treefrog

C

D

100 ms

	A	**B**	**C**
8 ms			
7 ms			
6 ms			
5 ms			
4 ms			
3 ms			
2 ms			
1 ms			
0.75 ms			
0.5 ms			
0.25 ms			

0.5 uV

5 ms

P1

0.25 μV

2.5 ms

Figure showing Amplitude (dB re 1 nV) versus Modulation rate (Hz) for three panels: low carrier frequency, middle carrier frequency, and high carrier frequency. Legend: ■ Gray treefrog, □ Green treefrog. X-axis values: 12.5, 25, 50, 100, 200, 400, 800.

A

B

- ■ Gray treefrog
- □ Green treefrog

- ● Female
- ○ Male

% Recovery

ICI (ms)

A

low carrier frequency   middle carrier frequency   high carrier frequency

Amplitude (dB re 1 nV)

- ● Female
- ○ Male

B

low carrier frequency   middle carrier frequency   high carrier frequency

Amplitude (dB re 1 nV)

- ● Female
- ○ Male

Modulation rate (Hz)