

Middle Age, a Key Timepoint for Changes in Birdsong and Human Voice.

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1 **Abstract**

2 Voice changes due to natural aging and neurodegenerative diseases are prevalent in the aging
3 population and diminish quality of life. Most treatments involve behavioral interventions that target the
4 larynx because of a limited understanding of central brain mechanisms. The songbird offers a unique
5 entry-point into studying age-related changes in vocalizations because of a well-characterized neural
6 circuitry for song that shares homology to human vocal control areas. Previously, we established a
7 translational dictionary for evaluating acoustic features of birdsong in the context of human voice
8 measurements. In the present study, we conduct extensive analyses of birdsongs from young, middle
9 and old male zebra finches. Our findings show that birdsongs become louder with age and changes in
10 periodic energy occur at middle age but are transient; songs appear to stabilize in old birds.
11 Furthermore, faster songs are detected in finches at middle age compared to young and old finches.
12 Vocal disorders in humans emerge at middle age, but the underlying brain pathologies are not well-
13 identified. The current findings will motivate future investigations using the songbird model to identify
14 possible brain mechanisms involved in human vocal disorders of aging.

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20 **Key Words: voice, speech, birdsong, zebra finch, aging**

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28 **1. Introduction**

29 The current population of individuals over 65 years of age in the United States is approximately 50
30 million people and will comprise 20% of the total population by 2060 (Mather, Jacobsen et al., 2015).
31 Voice disorders are present in at least 20-30% of this population and prevalence increases with
32 advancing age (Golub, Chen, Otto, Hapner, & Johns, 2006; Roy, Merrill, Gray, & Smith, 2005; Roy, Kim
33 Courey, & Cohen, 2016). Age-related voice changes, known as presbyphonia, encompass a range of
34 symptoms, including a hoarse, breathy and weak voice, vocal tremor, decreased intensity (perceived as
35 decreased loudness) and altered fundamental frequency (f_0 , perceived as pitch, Casper & Colton,
36 2000; Martins, Goncalvez, Pessin, & Branco, 2015). These voice deficits can co-occur with a decline in
37 accuracy and timing of speech movements that manifests as slow and less intelligible speech in elderly
38 subjects (Bilodeau-Mercure et al., 2015; Skoog Waller, Eriksson, & Sörqvist, 2015). Hearing loss from
39 destroyed hair cells and/or deficits in auditory-motor processing can also contribute to disordered
40 speech (Panouillères & Möttönen, 2018). Communication deficits diminish quality of life through
41 avoidance of social interactions, altered mental health and decreased work productivity when
42 individuals stay employed past retirement age (Roy, Merrill, Gray, & Smith, 2005; Yorkston, Bourgeois,
43 & Baylor, 2010). Most research investigations into the pathophysiology of aging voice as well as
44 treatments have focused on the periphery, by studying laryngeal structure and the acoustic product in
45 typical aging and in disease states.

46 In middle-aged and elder adults, typical aging voice abnormalities are associated with deterioration
47 in connective tissue, mucosa, cartilage and intrinsic muscles of the larynx. Structural changes cause
48 the vocal folds to lose their elasticity and stiffen, which, in conjunction with decreased respiratory drive
49 and possibly muscle mass, can lead to incomplete closure of the vocal folds and abnormal voice quality
50 (select reviews: Rapoport, Meiner, & Grant, 2018; Rosow & Pan, 2019). Consistent with research
51 focusing on the periphery, current treatments for aging voice target altering laryngeal structure, mass,
52 and strength (Gartner-Schmidt & Rosen, 2011; Mau, Jacobson, & Garrett, 2010). Unfortunately, vocal
53 deficits often continue to be reported at discharge. One possible explanation is that current treatments
54 cannot fully overcome limitations to peripheral mechanisms and another is that other, more central

55 mechanisms are important contributors to age-related dysphonia. The neurobiological mechanisms that
56 contribute to voice and speech deficits in the aging population are poorly understood, limiting treatment
57 options and motivating research investigations in animal models of vocal behavior. Neuropathological
58 findings from rodent models of normative aging implicate neuronal cell loss in the brainstem in
59 laryngeal dysfunction, but the contribution of higher brain centers are not known (Basken, Connor, &
60 Ciucci, 2012; Peterson, Watts, Morris, Shelton, & Cooper, 2013; Schwarz, Thompson, Connor, &
61 Behan, 2009; reviewed in: Lenell, Kelm-Nelson, Ciucci, & Johnson, 2018). With age-related
62 neurodegenerative diseases such as Parkinson's disease, deficits in dopamine modulation of cortico-
63 basal ganglia circuits contribute to vocal and limb motor deterioration in rodent models (Grant, Richter
64 et al., 2014; Grant, Barnett, Doll, Levenson, & Ciucci, 2015). One major limitation, however, in using
65 rodent models is the lack of well-characterized cortical and basal ganglia vocal circuitry which limits the
66 ability to identify underlying neural mechanisms.

67 By contrast, we advocate that songbirds are advantageous models for studying the impact of
68 normative and disease-related aging on the vocal neural circuitry (Austad, 2011; Miller, Hafzalla,
69 Burkett, Fox, & White, 2015). The male zebra finch (*Taenopygia guttata*) neural circuitry for song is well
70 described and shares considerable anatomical, genetic and circuit-level homologies to the human vocal
71 circuitry (Pfenning et al., 2014; Simonyan, Horwitz, & Jarvis, 2012). As is the case for humans,
72 auditory-guided feedback is used to maintain the learned vocalizations in finches; deafening leads to
73 vocal (e.g. song) deterioration (Brainard & Doupe, 2000; Lombardino & Nottebohm, 2000; Nordeen &
74 Nordeen, 1992). Quantification of birdsong can be made by using several measures common to human
75 voice studies including vocal intensity, f_0 , and Cepstral Peak Prominence (CPP), which provides
76 information about harmonic and noisy energy in sounds (Badwal, Poertner, Samlan, & Miller, 2019;
77 Hillenbrand & Houde, 1996). Wiener Entropy (WE) is a measure of spectral entropy in birdsong which
78 provides information both similar to and distinct from CPP (Badwal, Poertner, Samlan, & Miller, 2018).
79 Timing is quantified by measuring song duration and considering the silent intervals 'gaps' located
80 within and in-between songs (Chakraborty et al., 2017; James & Sakata, 2014). In human subject
81 research, timing is reflected in measurements of speaking or articulatory rate where speaking rate

82 includes the silent intervals known as pauses (Kuo & Tjaden, 2016). Speaking/articulatory measures
83 show greater fluctuations among elderly human subjects with age (Harnsberger, Shrivastav, Brown,
84 Rothman, & Hollien, 2008; Linville, 1996; Skoog Waller, Eriksson, & Sörqvist, 2015). Variations in song
85 timing, intensity, f_0 , and WE measures within the bird's song are actively generated by premotor neural
86 activity in finch cortical and basal ganglia song control nuclei (Day, Kinnischtzke, Adam, & Nick, 2008;
87 Hahnloser, Kozhevnikov, & Fee, 2002; Kao & Brainard, 2005; Kojima, Kao, & Doupe, 2013; Sober,
88 Wohlgemuth, & Brainard, 2008). Dopamine action on Area X, a vocal control basal ganglia nucleus,
89 can provide descending modulation onto the syrinx to actively vary f_0 and WE at the level of individual
90 syllables within the finch's song (Goller & Riede, 2013; Leblois, Wendel, & Perkel, 2010; Leblois &
91 Perkel, 2012; Miller, Hafzalla, Burkett, Fox, & White, 2015). By measuring changes in these acoustic
92 features over the finch lifespan and novelly relating them to human vocal measures, we can model
93 voice and speech changes in aging humans to lay the foundation for identifying the neurobiological
94 targets.

95 The aim of the current study was to examine whether aging differentially affects multiple acoustic
96 features in three distinct syllable types (noisy, harmonic or mixed) within the bird's song. We use a
97 cross-sectional comparative analyses across a narrowly defined age range of young, middle and old
98 adult male zebra finches (see Methods: Subjects). Notably, middle-aged finches in our study here were
99 defined as a separate age range from young and old finches, given the important consideration that
100 voice changes in f_0 among other features begin to emerge in middle-aged humans (~40-50 years of
101 age; Raj, Gupta, Chowdhury, & Chadha, 2010; Stathopoulos, Huber, & Sussman, 2011; Watts,
102 Ronshaugen, & Saenz, 2015). Only one prior study, conducted in a small number of Bengalese finches
103 (*Lonchura striata domestica*), identified a middle-aged group and compared it to older birds yet their
104 age ranges were distinct from ours (Cooper et al., 2012). Two other studies used a longitudinal
105 experimental design that tracked acoustic feature changes within a young and older adult group of
106 Bengalese/zebra finches over multiple recording periods (James & Sakata, 2014; Pytte, Gerson, Miller,
107 & Kirn, 2007). Our work builds from these previous studies with comparison to their varied results
108 forming the platform for our discussion. Based on the human literature on aging voice and speech, we

109 hypothesized that changes in intensity, degree of periodicity (e.g. CPP, WE, HNR), f_0 and timing will
110 begin to emerge at middle age in our data set with more progressive declines in these features
111 detected in older adult zebra finches. Our clear results provide a solid foundation to justify the use of
112 the songbird model in future studies to investigate age-related changes in vocal neural circuitry.

113 **2. Materials and Methods**

114 **Subjects:** All animal use was approved by the Institutional Animal Care and Use Committee at the
115 University of Arizona. Adult male zebra finches raised in aviary colonies were moved to individual
116 sound attenuation chambers for song recording purposes (Eckel Noise Control Technologies,
117 Cambridge, MA). Finches were acclimated for two days under a 13:11 h light:dark cycle before
118 beginning recordings. Finches between 1147 – 1402 days post-hatch (dph, n=10 finches) were
119 assigned to the ‘Old’ group. In our colony at the University of Arizona, established in 2014, our oldest
120 finches are ~1600 dph (4.4 years). Middle-aged birds in our colony were those at the midpoint in their
121 lifespan, recorded between 627-898 dph (n=8 finches). Syllable data from three of the eight middle-
122 aged birds was included in a prior, non-age-related study comparing acoustic measurements made in
123 birdsong with human voice in order to develop a ‘translational dictionary’ (Badwal, Poertner, Samlan, &
124 Miller, 2019). ‘Young’ adult finches were between 241 – 294 dph (n=8 birds). The tight distribution of
125 finch ages within the young, middle and old age groups enabled us to obtain more power in our
126 between-group comparisons and is a departure from other behavioral studies using a broader
127 distribution of ages and/or longitudinal recording periods (Cooper et al., 2012; James & Sakata, 2014;
128 Pytte, Gerson, Miller, & Kirn, 2007).

129 **Song Recordings:** Methods followed those of Miller et al. (2015). For the recording period, males
130 were housed alone in sound attenuation chambers and ‘undirected song’ (UD), a form of vocal practice
131 in which the male is alone, was recorded following lights-on in the morning. UD song was selected for
132 this experiment since the long-term goal of the work is as a model for human voice and speech
133 production. In human voice research, it is common to evaluate voice production of individual subjects
134 sustaining vowels or reading a passage (Patel et al., 2018). Conversational speech between two
135 people is less frequently used as a stimulus for laboratory measurement. UD songs show more

136 rendition to rendition variation in acoustic features, among them f_0 , compared to female-directed song
137 performance elicited by placing a female in the same cage (Kao, Doupe, & Brainard, 2005; Miller,
138 Hafzalla, Burkett, Fox, & White, 2015). Two hours of UD song was collected for all birds using Shure 93
139 lavalier condenser omnidirectional microphones connected to an audiobox (Audiobox: 44.1 kHz
140 sampling rate/24bit depth). When singing UD song, male finches tend to stay stationary in their cage
141 based on video recordings; our previous observations noted only a one decibel change in sound
142 intensity detected by the microphone if the bird was at the far regions of the cage. The intensity level of
143 each chamber was calibrated by playing a 1000 Hz tone in the chamber and measuring the intensity at
144 the microphone using a Type II Sound Level Meter (Galaxy Audio, Wichita, Kansas). The intensity of
145 calibration tone was measured in Praat and subtracted from the actual intensity to derive a calibration
146 factor. All measured intensities were later corrected using the calibration factor specific to the chamber
147 in which the song was recorded. Sounds were recorded and digitized using pre-set parameters for
148 capturing zebra finch song in the freeware program Sound Analysis Pro (SAP,
149 <http://soundanalysispro.com/> - Tchernichovski, Nottebohm, Ho, Pesaran, & Mitra, 2000).

150 **Song Analyses:** Song output in finches is represented by a series of motifs that are repeated over
151 time. Each motif contains a sequence of syllables comprised of harmonic, mixed or noisy notes (Fig. 1).
152 The 'mixed' syllables are comprised of features of both components (Figs. 1-2). Acoustic analyses of
153 birdsong are conducted using multiple measurements made at the syllable and motif level. Twenty-five
154 consecutive renditions (e.g copies) per bird were analyzed from the start of the UD song recording
155 period based on prior rationale (see section 3, Statistical Analysis). The most common motif
156 presentation of each bird was selected. The motifs across birds comprised a range of two to nine
157 unique syllables. Syllables were identified as distinct sound envelopes bounded by silence; the length
158 of silence varied across birds and within the motif across birds. The silence periods between syllables
159 were measured and included in the song rate calculations (see motif level analyses). Only the first five
160 syllables of each bird's motif were analyzed for acoustic features at the syllable level to ensure that
161 statistical analyses were not abnormally weighted towards birds with more syllables in their motifs. The
162 first occurrence of the syllable in the motif was included in the analyses. Song data was segmented in

163 motifs and by individual syllables in Praat (Badwal, Poertner, Samlan, & Miller, 2018; Boersma & Van
164 Heuven, 2001). Introductory notes and unlearned calls were excluded from the acoustic analyses.

165 **Praat:** The procedures for acoustic analysis are detailed by Badwal et al. (2019). In brief, a window
166 length of 0.025 seconds (s) and dynamic range of 70 dB were used. Mean intensity and smoothed CPP
167 were computed for all analyzed syllables. Mean intensity and CPP were calculated using standard
168 methodology as described in the Praat instruction manual included with the program. CPP is a robust
169 and frequently-used measure of overall voice quality that provides information about acoustic waveform
170 periodicity (Hillenbrand & Houde, 1996). A higher CPP value means a greater degree of periodicity in
171 the voice/birdsong. A pitch floor setting of 300 Hz and maximum frequency of 20,000 Hz were used in
172 generating the power cepstrogram to accommodate the high f_0 of birds. The Praat default settings were
173 otherwise used. The peak search range for “get CPPS” was also modified to accommodate typical finch
174 f_0 , and set to 300 to 1500 Hz. Syllable duration was measured by identifying the beginning and end of
175 distinct sound envelopes visually on the spectrogram shown in Praat. Glottal pulses and audio playback
176 were used as guidelines for measurement.

177 We followed the convention in birdsong analysis whereby fundamental frequency (f_0) is only
178 measured for harmonic syllables (Kao, Doupe, & Brainard, 2005). Harmonic syllables are comprised of
179 only one note in the form of flat harmonic stacks with relatively stable periodicity (e.g. Fig. 1B-syllable
180 E, Fig. 1C-syllables B, E). The f_0 mean, median and Harmonics to Noise Ratio (HNR) were not
181 calculated for any syllables that have ‘noisy components’ or ‘mixed components’ (e.g. Fig. 1A, syllables
182 B-D) because, by definition, they lack a consistent f_0 . For frequency analysis, we specified a 75 to 1600
183 Hz range and used the cross-correlation analysis option. Mean f_0 , standard deviation, minimum and
184 maximum f_0 , were measured using the “voice report” function in Praat for each harmonic syllable (Praat
185 manual). The f_0 range was computed as the difference between maximum and minimum f_0 , in Hz.

186 **Praat, motif level analyses:** Analysis of song timing was completed using the full motifs in order to
187 accurately characterize each bird’s production rate. We calculated two measures, “song rate” and
188 “articulation rate,” in this analysis. These measures are both calculated as number of syllables per unit

189 of time (syllables per second-SPS), normalizing the tempo for comparison across birds who produce
190 different numbers of syllables in their motif. To calculate 'song rate,' the number of syllables in the motif
191 was divided by the total motif duration, which was measured as the difference in time between the
192 beginning and end of the motif determined from a display showing the waveform and spectrogram.
193 Articulation rate was also calculated by dividing the number of syllables by total motif duration but total
194 pause time was subtracted from the motif duration in this calculation. Pauses between individual
195 syllables were identified as the silent periods (i.e. no sound energy). Pauses were measured
196 individually and added together to determine the total pause time for the motif.

197 **Song Analysis Pro (SAP):** Twenty-five copies of each syllable per bird were exported from Praat
198 as .wav files. Wav files for each syllable copy were run through the Sound Analysis Tools for Matlab
199 (<http://soundanalysispro.com/matlab-sat>) to obtain WE and Duration Scores. SAP outputs WE scores
200 on a logarithmic scale from zero to minus infinity where white noise is $\log(1) = 0$ and complete order is
201 $\log(0) = \text{minus infinity}$ ([http://soundanalysispro.com/manual-1/chapter-4-the-song-features-of-](http://soundanalysispro.com/manual-1/chapter-4-the-song-features-of-sap2/wiener-entropy)
202 [sap2/wiener-entropy](http://soundanalysispro.com/manual-1/chapter-4-the-song-features-of-sap2/wiener-entropy)). Harmonic syllables show low frequency modulation (FM) and WE values
203 approach negative infinity. Noisy syllables show high FM and WE values closer to zero (or white noise).

204

205 **3. Statistical Analysis**

206 Statistical analyses were performed using the SPSS statistical software package (IBM Corp.
207 Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY). Raw scores for each
208 feature were run using a General Linear Mixed Model (GLMM) in SPSS. Residuals generated from the
209 GLMM for each feature were examined using histograms to verify their symmetrical distribution and
210 validate conformity to the model. Residuals were fairly symmetrical with occasional skew deviating from
211 normal in which case we confirmed that a log transformation of the data yielded the same statistical
212 results from the GLMM including symmetrical residuals. The GLMM is well suited for dealing with the
213 presence of multiple, non-independent data points per bird and is often used for modeling behavioral
214 data, including birdsong (Hauber, Cassey, Woolley, & Theunissen, 2007; James & Sakata, 2014;
215 McCulloch & Neuhaus, 2005; Ripmeester, Kok, van Rijssel, & Slabbekoorn, 2010). Here, our

216 dependent variables were raw scores from 25 copies of each syllable per bird yielding 2,925 scores for
217 age-related changes in CPP, WE, intensity, and duration of all syllable types and 675 scores for
218 measurements of f_0 median, mean, range and HNR for the harmonic syllables only. At the motif level,
219 the total number of observations were 650 scores for measurement of song and articulatory rates. Our
220 prior studies showed that 25 syllable or motif copies enable detection of significant differences by age
221 or experimental treatment (Miller, Hilliard, & White, 2010; Miller, Hafzalla, Burkett, Fox, & White 2015).
222 No appreciable increase in power has been previously observed by the authors in any statistical test
223 when conducted on an $n \geq 25$ syllables/motifs in a given behavioral condition sung in a two-hour
224 recording period based on power calculations (Miller, Hilliard, & White, 2010). By not collapsing all data
225 to a single mean score per bird, we take into account natural variance in syllable and motif-level scores
226 over song renditions and improve the precision and power of our data in detecting a meaningful age
227 effect. Thus, to quantify between-bird variance, bird identity is specified as a 'random effect' in our
228 GLMM. The 'fixed effects' in the model are represented by the interaction of the data with two
229 experimental factors, Syllable Type and Age, where age is categorical (e.g. young, middle or old).

230 The statistical output from GLMM is represented in Tables 1-3 and in Figs. 3-7. Table 1 contains the
231 F-values, degrees of freedom and p-values from the GLMM 'Type III Tests of Fixed Effects'. For the
232 post-hoc pairwise comparison tests in Table 1, a Bonferroni correction was applied to obtain a new
233 alpha-level for significance based on $p=0.05/k$ ($k=9$ tests for age with syllable type or $k=3$ for age with
234 motif scores only). Because the Bonferroni correction results in a very small alpha level, Type II errors
235 (e.g. false negatives) can occur although this was offset somewhat by the statistical power of the
236 GLMM (see Results). Tables 2-3 list the Mean and 95% Confidence intervals (Lower Bound, Upper
237 Bound) data from the GLMM output for all acoustic features which were then plotted in the Origin
238 graphing program (version 2019). Supplemental Tables S1-S2 are SPSS files that contain all of the raw
239 syllable and motif data used for these calculations as well as the residuals output from the GLMM.
240 Furthermore, we compared 2,925 individual syllable scores for the raw WE data versus the raw CPP
241 values using the Bivariate function and Spearman's rho correlation test to determine whether they each
242 provide unique information about the birdsong, beyond just examining spectrograms (Fig. 2). Protocols

243 for using SPSS including syntax code for running song will be provided on our laboratory website:

244 <https://juliemiller.lab.arizona.edu/content/resources>.

245

246 **4. Results**

247 Figure 1 depicts exemplar motifs in spectrograms obtained from young, middle and old adult male
248 zebra finches; syllables are classified into three different types based on their sound energy - noisy,
249 harmonic and mixed. Letter identifiers are used to describe each unique syllable. In Fig. 1A, syllable A
250 is purely harmonic and syllable B noisy whereas syllables C and D are mixed because they are
251 comprised of both noisy and harmonic notes. By contrast, Fig. 1B illustrates a motif in which syllables
252 A-C are largely noisy but syllable E is a pure harmonic. In Fig. 1C, the motif is a mixture of all three
253 syllable types (noisy-A,C; harmonic-B,E; mixed-D). Fig. 2 depicts the CPP and WE scores for individual
254 syllables for the old bird shown in Fig. 1C. Harmonic syllables B and E have high CPP and low WE
255 scores (more negative) whereas noisy syllables A and C have lower CPP and higher WE scores (more
256 positive, e.g. closer to zero). Mixed syllable D scores do not fit these clear patterns – the CPP score is
257 closer to the noisy syllables and the WE score is similar to the harmonic syllables. In a previous study,
258 we hypothesized that CPP and WE scores appeared to be inversely correlated based on a small data
259 set from three middle-aged birds (Figs. 2-3 in Badwal, Poertner, Samlan, & Miller, 2019). Here, we use
260 a larger dataset from birds across different ages to examine the relationship between CPP and WE
261 further. CPP and WE scores show a weak, but significant inverse correlation based on a Spearman's
262 rho test performed on the raw scores indicating that they can provide somewhat distinct information
263 (N=2925, correlation coefficient: -0.220, 2-tailed, $p < 0.001$).

264 Each acoustic feature was evaluated based on its interaction with the syllable type (harmonic, noisy,
265 mixed) and the age of the bird (young, middle, old; Tables 1-3). Middle-age was a key time point for
266 changes in CPP and WE scores. Harmonic and mixed syllables showed a drop in CPP at middle age
267 compared to young and old adult ages indicating less periodic energy in the song (Fig. 3, Tables 1-2).
268 Noisy syllables did not show a significant change in CPP with age (Fig. 3). WE scores for mixed
269 syllables also showed a noticeable drop at middle-age, becoming more negative indicating greater

270 periodic energy compared to the young age group (Fig. 4, Tables 1-2). WE scores appear to rise again
271 for mixed syllables in old age, but the difference was not significant in *post hoc* tests because the p-
272 value (0.038) did not meet the low alpha-level (e.g. $p < 0.006$) for the Bonferroni correction. Similarly,
273 harmonic and noisy syllables also showed a trend for WE scores to either drop or rise at middle-age
274 compared to old age but the p-values (0.017, 0.042) did not meet the Bonferroni corrected p-value.

275 The intensity ('loudness') of the bird's song significantly increased with age in *post-hoc* tests
276 between young and middle-age and young versus old age for all three syllable types (Fig. 5, Tables 1-
277 2). The differences in intensity from middle-age to old-age were not significant, with p-values for the
278 *post-hoc* comparison tests between 0.009-0.011. The corrected alpha-level for this test was $p = 0.006$.
279 No significant age-related changes for f_0 mean, median, range and HNR scores were detected for
280 harmonic syllables (Tables 1,3). No main effect of age was detected for individual syllable duration
281 (Table 1).

282 At the motif level, both song/articulatory rates (with and without pauses, respectively) significantly
283 increased at middle age compared to young and old ages (Figs. 6-7, Tables 1,3). Rates were
284 calculated based on number of syllables/motif duration in seconds. Middle-aged finches did not have
285 fewer number of syllables in their motifs, but their motif duration (length) was significantly shorter
286 compared to old finches yet of a similar length to young finches (Tables, 1,3). The decreased motif
287 duration in the middle-aged group was not due a difference in the composition of the motif based on
288 syllable type. Mixed syllables, which are of longer duration than harmonic or noisy syllable type, were
289 not underrepresented in the middle-aged group compared to young and old finches.

290

291 **5. Discussion**

292 Birdsongs were compared across young, middle and old adult male zebra finches to determine whether
293 spectral and temporal acoustic features change with age and how these data relate to aging human
294 voice/speech studies. Our results show that middle age is a key time point in birdsong with changes in:
295 (a) periodic energy (e.g. CPP, WE measures) in select syllable types, (b) a faster song tempo even

296 when considering intersyllable silent periods, and (c) vocal intensity, with middle-aged birds producing
297 louder songs than young adults. Contrary to our hypothesis, neither WE, CPP, nor rate worsened from
298 middle to old age. For example, CPP in older birds was indistinguishable from the young adult group,
299 even though it was lower in middle age. Intriguingly, CPP and WE scores did not affect all syllable
300 types equally at middle-age. CPP was lower for harmonic and mixed syllables in middle-aged finches
301 than at other ages, indicating more noise (e.g. less periodic energy). By contrast, WE scores became
302 less noisy (e.g. more periodic) but only for the mixed syllable type. The WE scores for the harmonic and
303 noisy syllable types approached but did not reach significance. Given the number of tests completed,
304 the Bonferroni correction led to a very small alpha level, and there is certainly the possibility of a Type II
305 error (i.e., false negative) affecting our results here.

306 The contradictions between the CPP and WE measurements for the mixed syllables at middle age
307 may reflect differences in how the two programs (e.g. Praat versus SAP) assess and average the
308 temporal variations in energy over the time course of the syllable when it has both harmonic and noisy
309 components. Further, despite the power of evaluating 25 copies of each syllable type per bird, we were
310 still limited by the number of mixed syllables available to study. Mixed syllables were absent in several
311 finches per age group, and noisy syllables dominate the birds' motifs. Future investigations, therefore,
312 need to incorporate birds with higher numbers of mixed syllables to determine if the differences
313 between the CPP and WE findings hold. The correlation of CPP and WE across syllable types was only
314 22% (Spearman's rho: -0.22), supporting our earlier premise that CPP and WE provide useful yet
315 distinct information about age-related changes in sound periodicity (Badwal, Poertner, Samlan, & Miller,
316 2019).

317 Our results in zebra finch build and extend upon the few previously published studies on aging male
318 birdsong by using a narrower within-group age range for middle and old age finches, examining the
319 impact of age on three distinct syllable types and utilizing additional human voice measurements (e.g.
320 CPP, HNR, f_0 range). Cooper et al. (2012) compared two age categories, old versus middle-aged adult
321 Bengalese finches. Finches recorded at six years of age were classified as the old group ($n=4$; $\sim 2,190$
322 dph) and compared to finches recorded between one to three years old that were deemed the middle-

323 aged group (n=5; ~365 -~1,095 dph). Compared to the middle-aged finches, old finches showed
324 reduced vocal intensity (loudness) and a trend for reduced f_0 (pitch). Old finches also showed a slower
325 song tempo because of longer intersyllable silent intervals. When considering the source of the age-
326 related changes in Bengalese song, the authors did not find altered composition of ventral syringeal
327 muscle fibers between the old and middle age group, but additional factors must be considered such as
328 the myosin heavy chain composition which is dictated by usage (Adams & Elemans, 2019; Mead et al.,
329 2017).

330 The main significant findings of the Cooper et al. (2012) cross-sectional study, that Bengalese
331 finches have softer and slower songs by old age are opposite to our own results from zebra finches:
332 louder songs at middle and old age, and faster songs at middle age. The Bengalese finches showed a
333 trend for decreased pitch with age and the zebra finches did not. The difference in findings between the
334 studies may be explained by the age range used, species-specific differences in syringeal control and
335 the experimental design, including the number of data points analyzed. The old Bengalese finches were
336 almost twice as old as our zebra finches, and our middle-age group represented a much narrower
337 range in days post-hatch. Zebra finches use bilateral control of both left and right syringeal sound
338 generators simultaneously to produce most syllables (Goller & Cooper, 2004). By contrast, Bengalese
339 finches produce higher f_0 frequencies for harmonic syllables overall with more lateralized control; left
340 syringeal denervation can lead to a loss of spectral control of syllables (Elemans et al., 2015; Secora et
341 al. 2012; Srivastava, Elemans, & Sober, 2015). Thus, the lack of effect of aging on zebra finch f_0
342 frequencies may be due to the more bilateral representation of f_0 control in zebra finches versus
343 Bengalese.

344 Experimental design choices are likely contributing to the differences between our findings and two
345 additional studies of aging birdsong (James & Sakata, 2014; Pytte, Gerson, Miller, & Kirn, 2007). The
346 two studies involved longitudinal, rather than cross-sectional analyses. In the Pytte et al. (2007) study,
347 mean and standard deviation values were obtained at the motif and syllable level for 14 consecutive
348 renditions across three recording sessions over 11 months in adult male zebra finches categorized into
349 young and old age groups. In the young adult group, decreased variability for WE and f_0 scores

350 (measured as standard deviation) were detected over time and faster songs were due to shorter motifs.
351 The old age group showed no changes in these three acoustic features. James & Sakata (2014) used
352 Bengalese finches as they aged from young adults (range: 4-11 months, e.g. ~120-330 dph, n=22) over
353 a period of 6-47 months (mean: 17 months, e.g. ~510 dph). They sampled songs at different times of
354 the day whereas we sampled song immediately after lights-on in the morning. Though their study
355 included finches across a broader age range, our studies analyzed similar numbers of data points and
356 acoustic features, and used similar statistical procedures. No significant change with age was detected
357 for any of these spectral features in their study, although syllable types were not analyzed separately by
358 age with the exception of f_0 which was restricted to the harmonic syllables as done in our study. They
359 found that adult Bengalese finches had a faster song rate as they aged due to decreased duration of
360 intersyllable gaps, e.g. silent intervals. In our study, the zebra finches showed a faster song rate
361 regardless of whether the silent intervals were considered.

362 Based on the human literature, we predicted that changes in vocal quality, intensity and f_0 would
363 emerge at middle age in zebra finches and become exacerbated in old age. In aged humans, a drop in
364 the CPP and HNR indicate a noisier or breathier voice, and they are measures used to characterize
365 aging and disordered voices (Ferrand, 2002; Heman-Ackah, Michael, & Goding 2002; Hillenbrand &
366 Houde, 1996; Krom, 1995; Martin, Fitch, & Wolfe, 1995; Patel et al., 2018; Sauder, Bretl, & Eadie,
367 2017; Watts & Awan, 2011). Similarly, middle-aged finches may also show evidence of 'vocal disorder'
368 given their lower CPP compared to young and old adults. The source of the song changes in middle-
369 aged finches may be related to singing fatigue and/or impaired brain circuitry. Given that corticostriatal
370 plasticity pathways in the mammalian brain dysfunction in aged animals (reviewed in: Samson &
371 Barnes, 2013), we speculate that similar signaling deficits in the finch brain may be occurring at middle
372 age. Alternatively, it is possible that the faster and greater vocal intensity songs of middle age finches,
373 in comparison to the songs of the younger adults, represent song optimization for later courtship
374 performance. Female finches judge potential male mates partially based on song quality; they prefer
375 louder, faster and more complex songs (e.g. more unique syllables) although other factors also play a
376 role such as the beak color and similarity of the song structure to their paternal/sibling songs (Clayton,

377 1990; Collins, Hubbard, Houtman, 1994; Hauber, Campbell, & Woolley, 2010; Ritschard, Riebel, &
378 Brumm, 2010; Vyas, Harding, Borg, & Bogdan, 2009; Zann, 1996). Future investigations in this area
379 can examine whether the courtship songs of our middle age finches are more preferred by potential
380 female mates.

381 Contrary to human data, however, the lower CPP (e.g. noisier birdsongs) in middle-aged finches is
382 transient - the CPP scores naturally improved in the old age group. The reason for the improvement in
383 the CPP scores is unknown. It may be some process specific to this particular cross-sectional sample
384 or the continual singing (e.g. vocal exercise) over the lifespan that mitigates deficits in acoustic output.
385 Singing behavior and the amount of singing in finches can rapidly alter genes and molecular pathways
386 resulting in changes to neural activity patterns in vocally-dedicated sensorimotor brain areas within 30
387 minutes, two hours and with longer time courses (Hessler & Doupe, 1999; Hilliard, Miller, Fraley,
388 Horvath, & White, 2012; Jarvis & Nottebohm, 1997; Mello & Clayton, 1994; Mello & Ribeiro, 1998; So,
389 Munger, & Miller, 2019). Whether continual singing-driven exercise can explain the partial recovery of
390 CPP/WE scores in old finches is not known, but a longitudinal study underway might provide additional
391 information if the same pattern occurs. Singing has been shown to delay or decrease the effects of
392 aging on speaking voice in humans with effects on f_0 , intensity, etc (Lortie, Rivard, Thibeault, &
393 Tremblay, 2017; Prakup, 2012), but the neural and peripheral mechanisms involved are not known, in
394 contrast to finch studies.

395 In contrast to the improvement in CPP and WE scores from middle to old age, a progressive linear
396 rise in vocal intensity (perceived as loudness) of the birdsongs was detected with age for all syllable
397 types. The human data are more variable. Some studies have found that older adults show increased
398 intensity with age (Stathopoulos, Huber, & Sussman, 2011), while others showed decreased intensity
399 (Linville, Skarin, & Fornatto, 1989) or no difference in intensity with age (Biever & Bless, 1989; Huber,
400 2008; Sapienza & Dutka, 1996). Older adults also respond to cues to increase their intensity (Huber &
401 Spruill, 2008); in this case, the external cues prompting a male finch singing alone to increase his song
402 intensity are not known. In humans, age-induced increases in intensity might reflect hearing loss and
403 cognitive impairment in processing of complex acoustic signals due to changes in the auditory system

404 at multiple levels (e.g. outer hair cells, cochlear nerve, midbrain, cortex- Lin et al., 2013; Peelle &
405 Wingfield, 2016). The rise in vocal intensity with age in our finches is likely due to a combination of
406 factors. One driving source of the increased vocal intensity may be increased subsyringeal air sac
407 pressure, but the neural or environmental signal triggering this physical change is not known. Zebra
408 finches are known to increase their song intensity due to high background noise (Zollinger, Goller, &
409 Brum, 2011), but our males are recorded singing alone in quiet environmental conditions. Hearing loss
410 from either cochlear hair cells or central auditory processing errors are other potential sources of
411 increased vocal intensity with age. It is not known whether middle-aged or old finches have hearing
412 loss. If so, it is certainly possible that their song is louder so they can hear themselves or so that older
413 females can hear them (Ritschard, Riebel, & Brumm, 2010). Thus, future studies will evaluate the
414 central and/or peripheral mechanistic changes responsible for the increased vocal intensity with age.

415 We did not detect changes in f_0 or HNR in our male birdsongs despite the abundance of human
416 literature documenting age and sex-dependent differences in these measurements. In a study
417 conducted across the human lifespan, male participants showed a decline in f_0 from 4 to 50 years of
418 age and then a steady rise (Stathopoulos, Huber, & Sussman, 2011); declining testosterone may be
419 one contributing factor, as reviewed in Lenell, Sandage, & Johnson (2019). The lack of f_0 change in our
420 syllable data may reflect differences in functional morphology between avian and human vocal organs
421 or a low number of harmonic syllables in the motif for analysis. In avian species, the syrinx is comprised
422 of two independent sound generators on the left and right sides, supported by separate sets of muscles
423 and ipsilateral neural control (Riede & Goller, 2010; Elemans et al., 2015). The dual nature of the sound
424 generators and muscles in zebra finch may therefore prevent age-related declines in control of f_0 .
425 Alternatively, the ability to detect f_0 changes in birdsong with age in our data may be limited by the
426 number of harmonic syllables which occur only once or twice in a motif. Future directions in this
427 research could study age effects on female songbirds such as the Northern cardinal and house wren
428 (Odom & Benedict, 2018). By studying age-related changes in both female and male songbirds, one
429 can draw parallels with sex differences present in aging human voice (Dehqan, Scherer, Dashti, Ansari-

430 Moghaddam, & Fanaie, 2012; Goy, Fernandes, Pichora-Fuller, & van Lieshout, 2013; Lenell, Sandage,
431 & Johnson, 2019).

432 In addition to drawing comparisons between aging human voice and spectral features of the bird's
433 song, we also examined temporal changes and relevance to human speech. Speaking and articulatory
434 rates were defined in the finch song by the number of syllables divided by the motif duration with
435 pauses between syllables in the motif excluded from the articulatory calculations. In humans, speaking
436 rate is the number of syllables or words per unit of time with articulatory rate excluding pauses.
437 Interestingly, middle age finches produced songs at a faster rate in contrast to middle-aged human
438 adults who show slower articulatory rates compared to younger adults (Jacewicz, Fox, O'Neill, &
439 Salmons, 2009). Other studies examined human speaking/articulatory rates in young adults (ages 21-
440 32) versus older adults (66-90 years; middle-aged was not separately categorized; Bona, 2014) finding
441 that young adults spoke faster depending on the task. Elder adults in general show a natural decline in
442 speech rate (Gollan and Goldrick, 2019; reviewed in Linville, 1996). Our old finch group did not show a
443 decline in song/articulatory rates compared to young or middle-aged finches indicating that
444 compensatory mechanisms may be engaged to prevent song deterioration. It is clear that there is
445 considerable variation in the human data by age, and it is complicated by the fact that the articulatory
446 movements of the tongue, lip and jaw in coordination with the larynx direct sequence timing (Berry,
447 2011). In finches, potential articulators filtering the sound include the amount of beak gape and the
448 expansion of the oropharyngeal-esophageal cavity (Ohms et al., 2010).

449 Middle age appears to be a key time point for the emergence of age-related changes in vocal
450 function in both zebra finches and humans. The finch is an attractive model to study the effects of aging
451 on molecular and cellular interactions between the brain, syrinx and the vocal output across the
452 lifespan. Continued study using this zebra finch model can provide foundational knowledge about
453 central and peripheral mechanisms of aging and potentially lead to improved treatments for aging
454 human voice.

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457 **Figure Legends.**

458

459 **Figure 1. Motif exemplars from different ages and syllable types.** Audio waveform (top) and
460 spectrogram (bottom). Spectrogram is depicted as time (in seconds, x-axis) versus frequency (in Hertz,
461 y-axis). Color in the spectrogram represents the amplitude of the spectral energy at that frequency, with
462 red being the most intense followed by orange, yellow, and blue.

463 **A)** Motif from Young Bird (band identifier, Black 262) shown as a sequence of syllables (A–D). Syllables
464 are uniquely identified by letters and type assigned based on visual inspection and acoustic data.

465 A = harmonic, B = noisy, C, D = mixed.

466 **B)** Motif exemplar from Middle-Aged Bird (band identifier, Black 387) shown as a sequence of syllables
467 (A-E). A-C = noisy; D=mixed, E = harmonic

468 **C)** Motif exemplar from Old Bird (band identifier, Purple 601) shown as a sequence of syllables (A-E).

469 A, C = noisy, B, E = harmonic, D = mixed

470

471 **Figure 2. Motif exemplar of syllable types with CPP and WE scores.** Motif exemplar from old bird
472 in Fig. 1C is shown here with raw CPP and WE scores reported below each syllable. CPP is higher for
473 harmonic syllables B and E compared with noisy syllables A and C. Mixed syllable D has a CPP score
474 closer to the noisy syllables. WE scores are lower for the harmonic syllables, closer to negative infinity.
475 By contrast, noisy syllables have WE values that are less negative, and therefore closer to a score of
476 zero with zero equal to white noise (Tchernichovski, Nottebohm et al., 2000). Mixed syllable D, which
477 has a dominant harmonic component, has a WE score that is similar to the harmonic syllables.

478

479 **Figure 3. CPP drops at middle age for harmonic and mixed syllables.** The acoustic feature is
480 treated as the dependent variable and plotted on the y-axis with age category and/or syllable type
481 plotted on the x-axis. Cepstral Peak Prominence (CPP) is measured in decibels of Sound Pressure
482 Level (db). Mean values are represented as circles and error bars represent the lower (minus) and
483 upper (plus) 95% confidence intervals. Both harmonic and mixed syllable types show a significant drop

484 at Middle Age compared to Young and Old Finches (*Table 1). Noisy syllables do not show a significant
485 change in CPP across the three age groups. Data obtained from Table 2.

486

487 **Figure 4. WE drops at middle age for mixed syllables.** Wiener Entropy (WE) values are plotted on a
488 scale from -1.4 to -3.6 where harmonic syllable scores are more negative approaching complete order
489 (a pure tone equals negative infinity) whereas noisy syllables are less negative indicating that their
490 structure is less ordered or more random (white noise = 0; Tchernichovski, Nottebohm et al., 2000).
491 Mixed syllables appear to have WE scores in-between harmonic and noisy syllables. Mixed syllables
492 show a significant drop in WE at Middle Age compared to Young and Old finches (*Table 1). Harmonic
493 syllables also show a trend for a similar drop at Middle Age. Noisy syllables show a trend for a drop at
494 Old Age. Mean values are represented as circles and error bars represent the lower (minus) and upper
495 (plus) 95% confidence intervals. Data obtained from Table 2.

496

497 **Figure 5. Song intensity increases with age.** The acoustic feature was treated as the dependent
498 variable and plotted on the y-axis with age category and syllable type plotted on the x-axes. The
499 intensity (loudness) of the song measured in decibels (db) gets significantly louder from young to
500 middle age for all three syllable types and between young and old age (*Table 1). Mean values are
501 represented as circles and error bars represent the lower (minus) and upper (plus) 95% confidence
502 intervals from Table 2 data.

503

504 **Fig. 6. Song rate increases at middle age.** Motif-level data is presented here across finches where
505 mean values in syllables/second (syll/sec, circles) were obtained by dividing the number of syllables by
506 the total motif duration, including silent intervals 'pauses' between syllables (Table 3). Error bars
507 represent the lower (minus) and upper (plus) 95% confidence intervals from Table 3 data. Middle aged
508 finches showed a significantly faster song rate compared to young and old finches (*Table 1).

509 **Fig. 7. Articulatory rate increases at middle age.** Motif-level data is presented here across finches
510 where mean values in syllables/second (syll/sec, circles) were obtained by dividing the number of
511 syllables by the total motif duration and subtracting the total pause time between syllables. Error bars
512 represent the lower (minus) and upper (plus) 95% confidence intervals from Table 3 data. Middle aged
513 finches showed a significantly faster articulatory rate compared to young and old finches (*Table 1).

514 **See Tables on the following pages.**

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Acoustic Feature	Df	F-value	P-value	Post-hoc pairwise comparison p-value	Bonferroni corrected alpha level
CPP with Age Syllable Type Age and Syllable Type	24 2884 2888	7.5 2428.9 28.0	$p<0.003$ $p<0.001$ $p<0.001$	Harmonics: M vs. Y, $p<0.001$; M vs. O, $p<0.001$ Mixed: M vs. O, $p<0.005$; M vs Y, $p<0.002$ Noisy: NS	$p<0.006$
WE with Age Syllable Type Age and Syllable Type	24 2904 2905	1.1 273.1 37.1	$p=0.353$ $p<0.001$ $p<0.001$	Harmonics: NS; Mixed: M vs Y, $p<0.004$; Noisy: NS	$p<0.006$
Intensity with Age Syllable Type Age and Syllable Type	23 2893 2893	25.0 38.8 2.4	$p<0.001$ $p<0.001$ $p=0.051$	Harmonics: M vs. Y, $p<0.001$; O vs Y, $p<0.001$ Mixed: M vs. Y, $p<0.001$ O vs. Y, $p<0.001$ Noisy: M vs. Y, $p<0.001$, O vs. Y, $p<0.001$	$p<0.006$
Duration with Age Syllable Type Age and Syllable Type	23 2906 2907	0.2 433.2 18.5	$p=0.795$ $p<0.001$ $p<0.001$	Harmonics vs Mixed vs Noisy, $p<0.001$ NS	$p<0.017$
f_0 median with Age Harmonics only	17	0.2	$p=0.838$		
f_0 mean with Age Harmonics only	17	0.2	$p=0.798$		
f_0 range with Age Harmonics only	17	0.7	$p=0.507$		
HNR with Age Harmonics only	17	0.3	$p=0.774$		
Song Rate with Age (#syllables/second, includes pauses)	647	25.0	$p<0.001$	M vs. Y, $p<0.001$; M vs. O, $p<0.001$; O vs. Y, NS	$p<0.017$
Articulatory Rate with Age (#syllables/second – total pauses)	647	39.6	$p<0.001$	M vs. Y, $p<0.001$; M vs. O, $p<0.001$; O vs. Y, NS	$p<0.017$
Motif Duration	647	8.1	$p<0.001$	M vs. O, $p<0.001$; O vs. Y, $p<0.005$, M vs Y, NS	$p<0.017$

531

532 **Table 1.** Statistical output for the acoustic features were generated from the SPSS General Linear
533 Mixed Model (GLMM) and reported here as degrees of freedom (df), the ANOVA F-values and p-

534 values. Significant p-values for Syllable CPP, WE, Intensity, Duration and Song/Articulatory Rates were
535 further assessed using *post-hoc* tests. For the *post-hoc* comparisons, any p-values less than the stated
536 Bonferroni corrected alpha level were considered significant. The Bonferroni corrected p-values are
537 based on an alpha level of 0.006 where $k=0.05/9$ tests (e.g. CPP, WE, Intensity, Duration) to assess
538 the interaction of age and syllable type together or, $k=0.05/3$ to assess age on the motif-level measures
539 (Song rate, Articulatory rate, motif duration). NS: not significant

540 **Tables 2-3 are on the next pages.**

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Acoustic Feature	Age	Mean	Lower Bound (95% CI)	Upper Bound (95% CI)
Syllable CPP (dB)				
Harmonic	Y	21.80	20.93	22.66
	M	18.39	17.54	19.24
	O	21.39	20.68	22.10
Mixed	Y	14.52	13.69	15.35
	M	12.51	11.63	13.38
	O	14.20	13.46	14.93
Noisy	Y	11.26	10.48	12.04
	M	11.26	10.49	12.04
	O	11.73	11.03	12.44
Syllable WE				
Harmonic	Y	-2.87	-3.16	-2.57
	M	-3.28	-3.57	-2.98
	O	-2.80	-3.05	-2.55
Mixed	Y	-2.26	-2.55	-1.98
	M	-2.90	-3.20	-2.61
	O	-2.49	-2.74	-2.23
Noisy	Y	-2.13	-2.41	-1.85
	M	-1.91	-2.19	-1.63
	O	-2.30	-2.54	-2.05
Syllable Intensity (dB SPL)				
Harmonic	Y	51.81	48.53	55.09
	M	60.74	57.44	64.01
	O	66.78	63.85	69.71
Mixed	Y	51.70	48.42	54.97
	M	60.81	57.53	64.09
	O	66.80	63.86	69.73
Noisy	Y	51.47	48.20	54.75
	M	60.60	57.32	63.88
	O	66.44	63.51	69.37
Syllable Duration (ms)				
Harmonic	Y	0.064	0.042	0.087
	M	0.068	0.045	0.090
	O	0.064	0.045	0.084
Mixed	Y	0.172	0.149	0.194
	M	0.155	0.133	0.178
	O	0.140	0.120	0.160
Noisy	Y	0.101	0.080	0.123
	M	0.086	0.064	0.107
	O	0.112	0.093	0.131

556

557 **Table 2. Means and 95% Confidence Intervals from SPSS GLMM.** Acoustic features for all syllable
558 types are shown for the output from the model as the Means, Lower Bound (LB) and Upper Bound (UB)
559 95% confidence interval values. Y-Young age, M-Middle-age, O-Old Age.

560

Acoustic Feature	Age	Mean	Lower Bound (95% CI)	Upper Bound (95% CI)
Syllable f ₀ median (Hz)	Y	635.67	559.52	711.82
Harmonic only	M	605.92	529.94	681.91
	O	625.67	572.17	679.18
Syllable f ₀ mean (Hz)	Y	632.52	556.32	708.72
Harmonic only	M	599.43	523.39	675.46
	O	622.98	569.44	676.53
Syllable f ₀ range (Hz)	Y	51.34	27.58	75.10
Harmonic only	M	55.21	31.49	78.94
	O	40.21	23.47	56.95
Syllable HNR (Hz)	Y	11.34	8.28	14.41
Harmonic only	M	9.94	6.87	13.00
	O	10.97	8.80	13.13
Song Rate with Age (#syllables/second, includes pauses)	Y	11.10	10.56	11.64
	M	13.28	12.74	13.82
	O	11.65	11.17	12.13
Articulatory Rate with Age (#syllables/second – total pauses)	Y	9.57	9.29	9.85
	M	11.24	10.95	11.52
	O	9.84	9.59	10.09
Motif Duration (length)	Y	0.69	0.66	0.72
	M	0.67	0.64	0.70
	O	0.75	0.72	0.77

561

562 **Table 3. Means and 95% Confidence Intervals from SPSS GLMM.** Acoustic features for harmonic
563 syllables and motif-level data are shown for the output from the model as the Means, Lower Bound (LB)
564 and Upper Bound (UB) 95% confidence interval values. Y-Young age, M-Middle-age, O-Old Age.

565

566 **Supplemental Table 1. Syllable data.** SPSS file represents all raw acoustic scores for copy numbers
567 1-25 for each syllable per bird. Columns from left to right: Age category (Y-Young, M-Middle, O-Old),
568 Age in days post-hatch (dph), Syllable Identifier (A-E), Syllable Type (Noisy, Mixed, Harmonic), Copy
569 Number (1-25) followed by raw scores for syllable acoustic features and residuals 'RESID' from the
570 GLMM model. For Intensity, additional columns include the sound chamber in which the bird was

571 recorded (1-6) and the corrected intensity, 'CorrIntensity,' values for each score based on calibration
572 (see Methods).

573 **Supplemental Table 2. Motif data.** SPSS file represents all raw acoustic scores for motif copy
574 numbers 1-25 per bird. Columns from left to right: Age category (Y-Young, M-Middle, O-Old), Age in
575 days post-hatch (dph), Motif copy number (1-25), Number of syllables, followed by the raw scores for
576 the motif acoustic features, pause times and residuals 'RESID' from the GLMM model. SPS – Syllables
577 Per Second.

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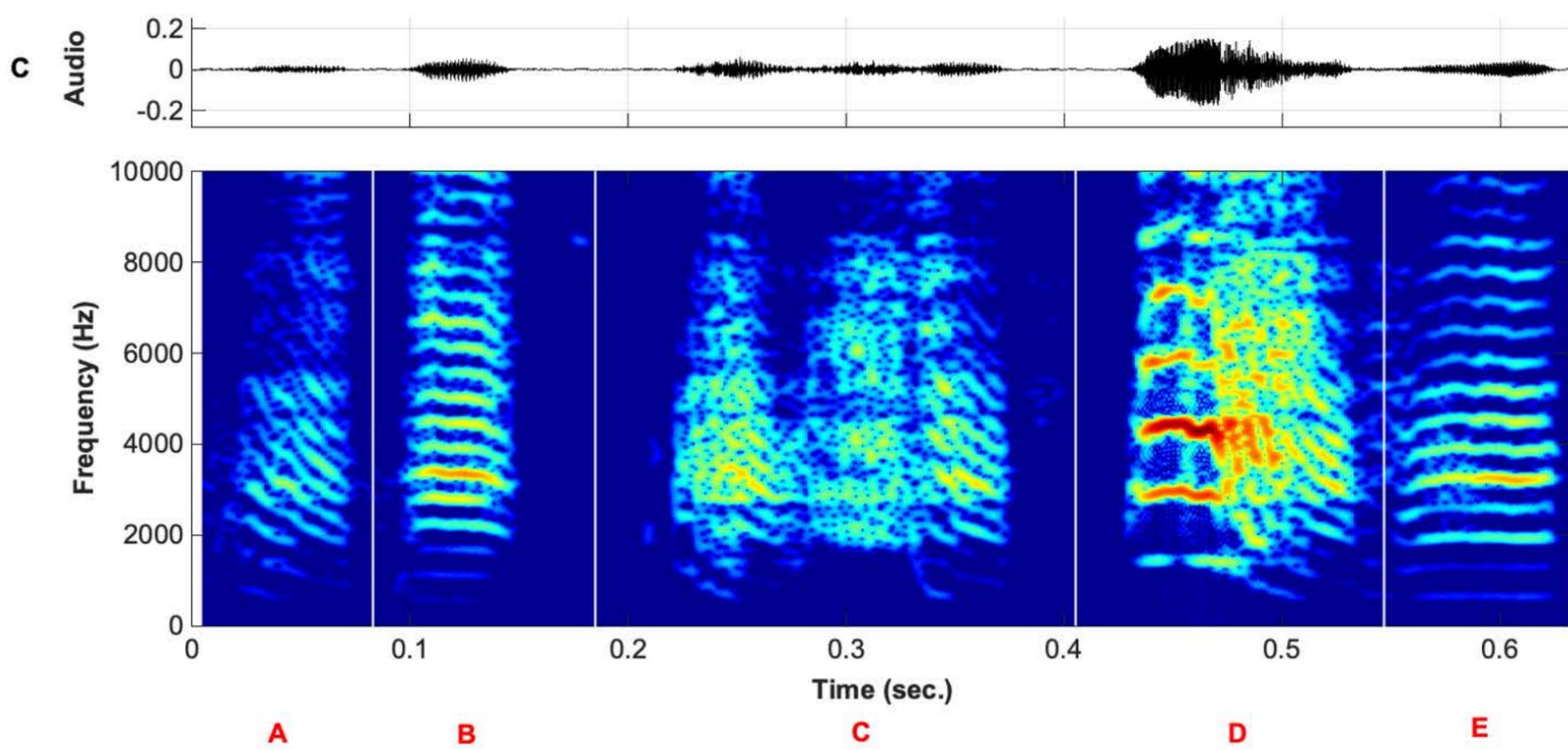
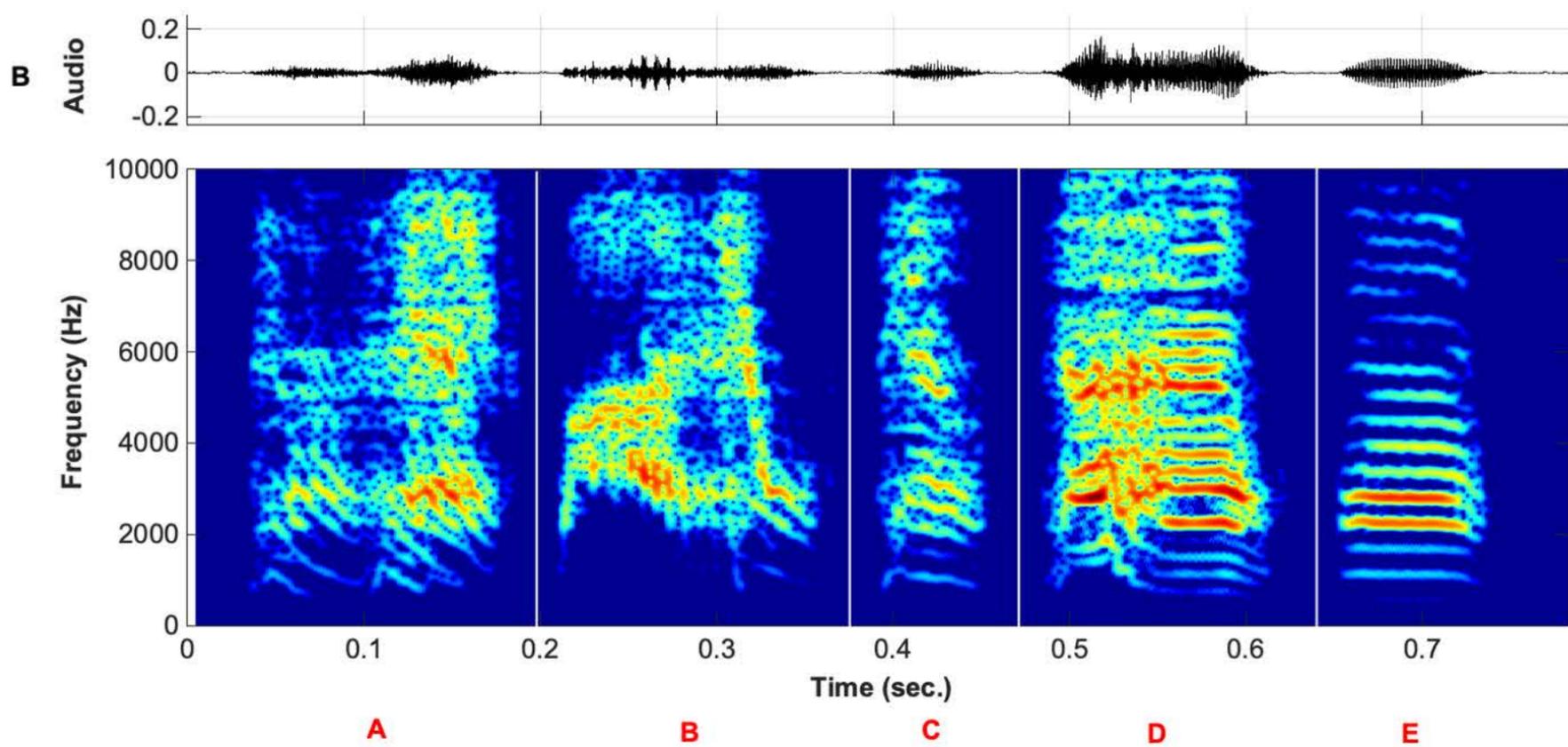
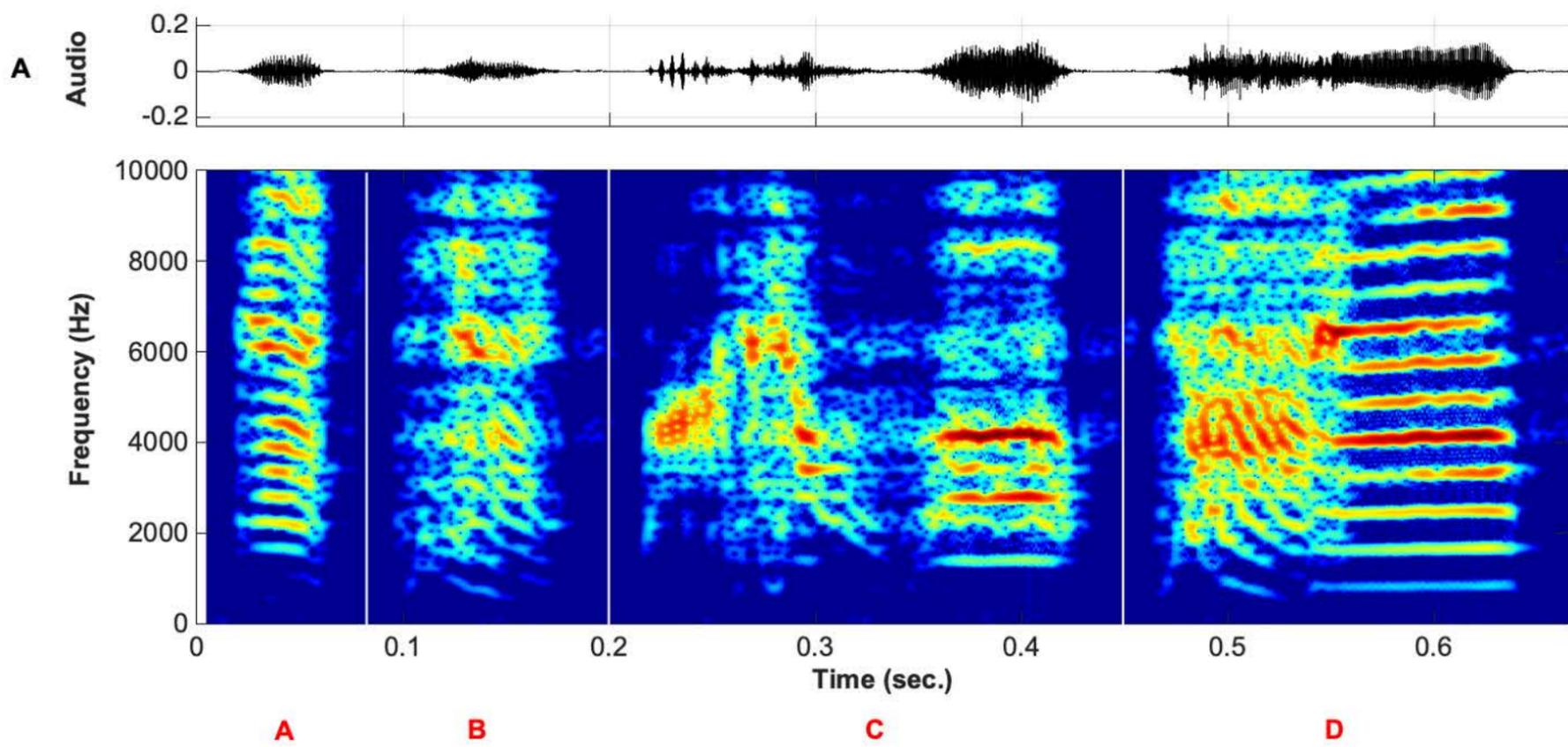
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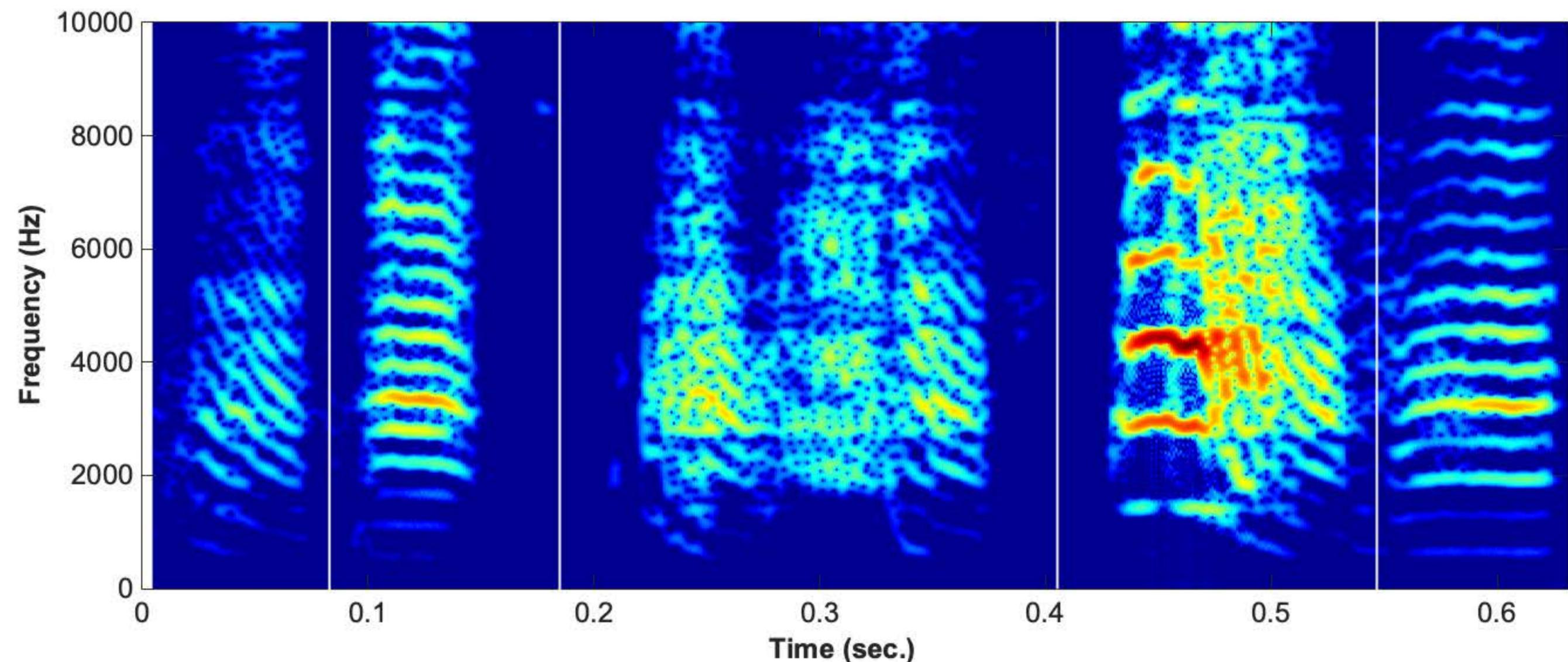
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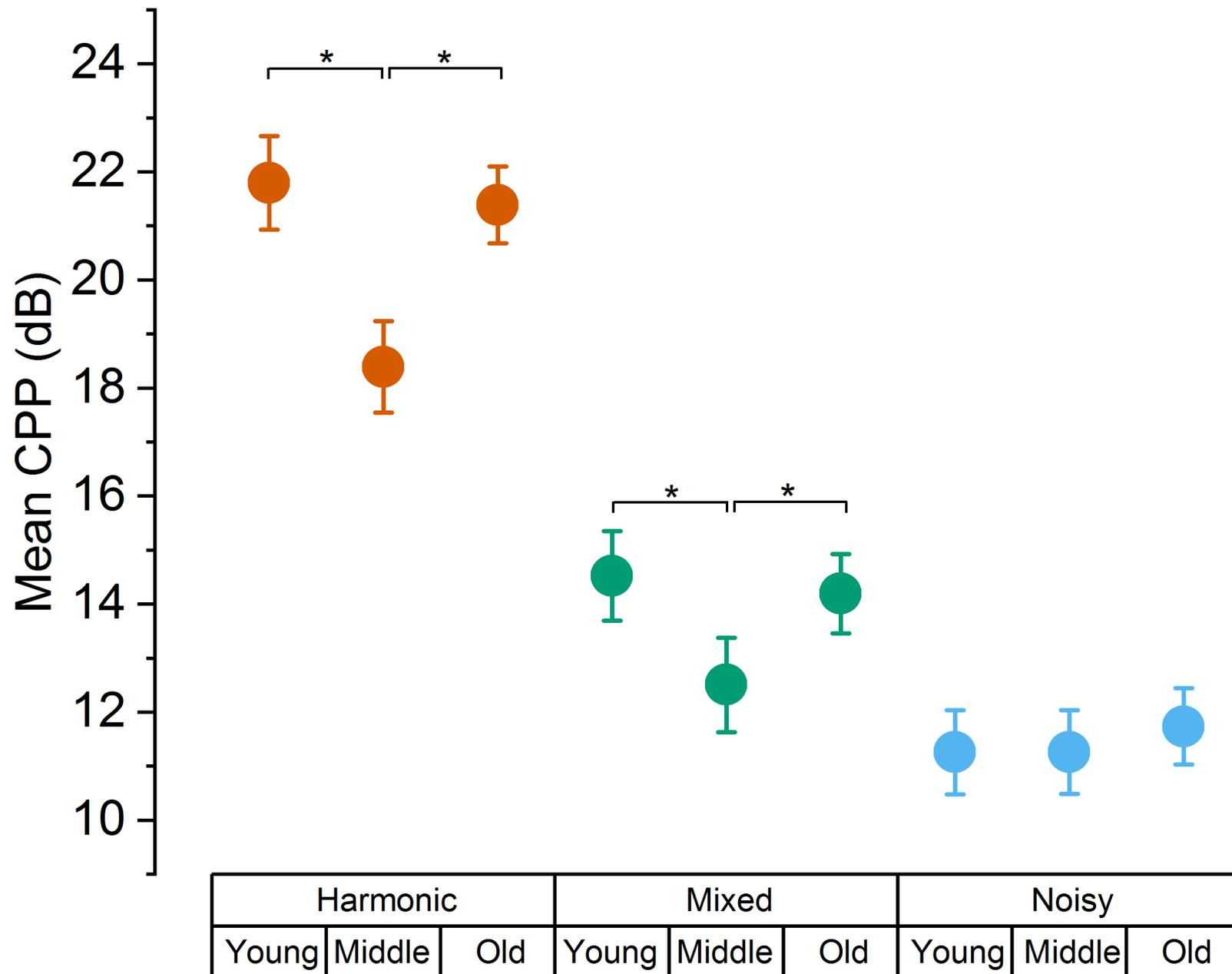
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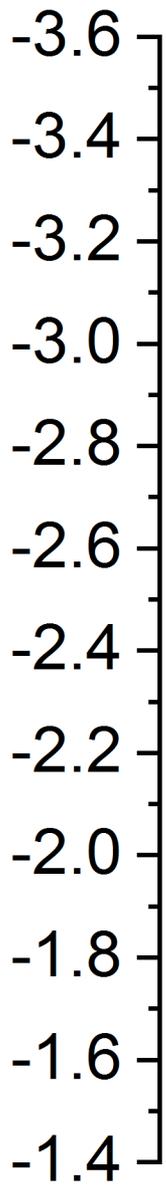




A	B	C	D	E
CPP = 12.8 dB WE = -1.72	CPP = 21 dB WE = -2.2	CPP = 10.4 dB WE = -1.79	CPP = 12.4 dB WE = -2.37	CPP = 20 dB WE = -2.76



Mean WE



Harmonic			Mixed			Noisy		
Young	Middle	Old	Young	Middle	Old	Young	Middle	Old

