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► To cite this version:

Alexandra C. Green, Cameron E.F. Clark, Sabrina Lomax, Livio Favaro, David Reby. Context-related variation in the peripartum vocalisations and phonatory behaviours of Holstein-Friesian dairy cows. *Applied Animal Behaviour Science*, 2020, 231, pp.105089 -. 10.1016/j.applanim.2020.105089 . hal-03492532

HAL Id: hal-03492532

<https://hal.science/hal-03492532v1>

Submitted on 22 Aug 2022

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1 **Context-related variation in the peri-partum vocalisations and phonatory**
2 **behaviours of Holstein-Friesian dairy cows**

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12

13 **Abstract**

14 **Context-related information** can be reflected within mammalian vocalisations and could in turn be
15 studied to benefit production animals. Whilst previous research has revealed **the contextual, and even**
16 emotional content of goat, pig and horse vocalisations, cattle vocalisations remain relatively less
17 explored. In this study we recorded the vocalisations and accompanying phonatory behaviours of 19
18 Holstein-Friesian dairy cows (n = 10 primiparous, n = 9 multiparous) during the **peri-partum contexts** of
19 parturition with dystocia and fence-line calf separation. Findings revealed that vocal structure was

20 context dependent, with parturition calls longer in duration, higher in fundamental frequency (F0) min
21 and F0 start, containing more nonlinear phenomena and more minor F0 modulations. By contrast, calls
22 during calf separation were distinguishable by their F0 contour, having a higher F0 mean and F0 max,
23 and greater F0 var, F0 cv and F0 absolute slope. During parturition, primiparous cows emitted calls
24 longer in duration, lower in F0 mean, and containing more F0 modulations than their multiparous
25 conspecifics. Parturient cows expressed a greater number of open mouth calls, whereas mixed calls
26 were more common in the calf separation context. Additionally, 82% of the primiparous and 10% of the
27 multiparous cow vocalisations involved tongue exposure from the mouth during parturition, which may
28 represent a visual indicator of compromised welfare. Overall, our results show that the **context-related**
29 **variation is conveyed through an integration of vocal and visual sensory modalities, with the**
30 **combination of an open mouth configuration, tongue protrusion, and spectral properties such as an**
31 **overall lower F0 and longer duration during parturition likely enhancing vocal transmission over longer**
32 **distances to inform more receivers about the urgency of this context.** Knowledge of these vocal and
33 behavioural cues could be adopted on farm to determine the welfare of peri-partum cattle and are
34 instrumental for future cattle bioacoustics research.

35

36 *Keywords:*

37 *Calf separation; parturition; vocal communication; welfare*

38

39 **1. Introduction**

40 There are many welfare challenges associated with the peri-partum period in cows (Rørvang et al.,
41 2018). Parturition is painful for many mammalian species, particularly the first time or when foetal

42 expulsion is difficult (Barrier et al., 2012; Mainau and Manteca, 2011; Remnant et al., 2017). Moreover,
43 in commercial farming environments, peri-partum cows may also be group housed to facilitate farm
44 management, and this can lead to mismothering and agonistic social interactions (Rørvang et al., 2018).
45 Within 24 hours post-partum, industry **common practice is** to separate the cow and calf to minimise the
46 bond formation that occurs soon after birth (Hudson and Mullord, 1977), as well as associated distress
47 responses from the cow (Flower and Weary, 2001; Lidfors, 1996; Stěhulová et al., 2008). Alongside this,
48 herd sizes are increasing and individual animal attention from farmers is diminishing (Barkema et al.,
49 2015). Thus, novel welfare assessment techniques are necessary to improve peri-partum farming
50 practices and alert farmers to those animals requiring attention.

51 The study of vocal behaviour presents an opportunity to assess an individual's attributes non-invasively
52 (Green et al., 2017). Mammalian vocalisations have been shown to contain dynamic cues to
53 motivational state (Morton, 1977), **context (Taylor et al., 2009)** and more recently, emotions, aligning
54 with the arousal/valence framework (Briefer, 2012; Scherer, 1986). Accordingly, there has been a surge
55 in bioacoustics research surrounding the emotional content of livestock vocalisations, especially of goats
56 (Baciadonna et al., 2019; Briefer et al., 2015b), pigs (Briefer et al., 2019; Leliveld et al., 2017; Linhart et
57 al., 2015), horses (Briefer et al., 2015a) and recently cattle (Green et al., 2019). **Cattle have been**
58 **recorded vocalising in the welfare-compromising contexts of** handling (Grandin, 2001), restraint and
59 branding (Watts and Stookey, 1999), and with differing methods of calf separation (Ikeda and Ishii,
60 2008; Weary and Chua, 2000). While some acoustic analyses have been undertaken to assess **context-**
61 **related variation** (Weary and Chua, 2000; Yeon et al., 2006), cattle vocal research has traditionally
62 focused on conspicuous vocal measures such as calling rate (Flower and Weary, 2001; Lidfors, 1996;
63 Stěhulová et al., 2008) and the quantification of high-frequency call types (Johnsen et al., 2015), rather
64 than the spectrographic features for a more comprehensive analysis of welfare state.

65 According to the source-filter theory of voice production (Fant, 1960), mammalian vocalisations
66 commence in the larynx (source of the sound) through vibrations of the vocal folds, whose rate
67 determines the fundamental frequency (F0), amplitude and duration of the sound (Taylor and Reby,
68 2010). This sound is subsequently filtered through the supra-laryngeal vocal tract (filter of the sound)
69 which is responsible for altering the formant frequencies and energy distribution of the sound (Taylor
70 and Reby, 2010). The source-filter framework has been adopted over the last few decades to investigate
71 static indexical and fluctuating motivational or emotional vocal cues in a range of mammalian species,
72 including deer (Reby and McComb, 2003), primates (Gamba et al., 2011), goats (Briefer and McElligott,
73 2011; Favaro et al., 2014), pigs (Briefer et al., 2019), and more relevantly cattle (Green et al., 2019;
74 Padilla de la Torre et al., 2015). Animal exposure to a stressor results in changes in respiration, salivation
75 and muscle tension through the triggering of the autonomic nervous system (Briefer, 2012). This in turn
76 results in the animal altering its source and/or filter-related vocal features (Briefer, 2012; Scherer, 1986).
77 Several F0-related vocal parameters have been associated with different production contexts (Mcgrath
78 et al., 2017; Taylor et al., 2009; Yin and McCowan, 2004) and further, source-related vocal parameters
79 including nonlinear phenomena, amplitude, F0 mean, F0 variability, and/or call duration have all been
80 shown to increase alongside increasing situation urgency (Koutseff et al., 2017; Marx et al., 2003;
81 Stoeger et al., 2012, 2011), likely mediated by changes in physiological arousal. Context-related vocal
82 modulations triggered by differences in emotions and/or motivations seem to be shared homologously
83 across mammalian species, likely due to commonalities in morphology, physiology and vocal production
84 mechanisms (Briefer, 2012). Thus, the application of the source-filter theory will be helpful in
85 deciphering the welfare-related context variation of cow vocalisations, along with their communicative
86 functions.

87 Here we investigated the acoustic differences of Holstein-Friesian cattle vocalisations, produced during
88 the peri-partum contexts of parturition with dystocia and fence-line calf separation. Moreover, both

89 primiparous and multiparous cows were observed to assess the effect of parity on peri-partum vocal
90 responses. The accompanying phonatory behaviours at the time of each vocalisation including mouth
91 configuration and tongue protrusion are also described, to determine the relationship between vocal
92 production and other conspicuous behavioural parameters. We hypothesised that there would be
93 context-related variability in the cow vocalisations and their underlying phonatory behaviours, which
94 may reflect differences in motivations and emotions that the cattle were experiencing.

95

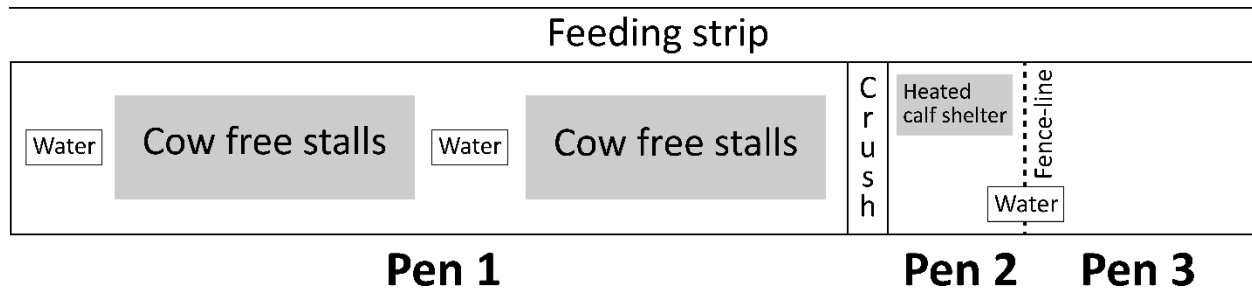
96 **2. Materials and methods**

97 *2.1. Animals and management*

98 The experiment was conducted in a maternity barn (Figure 1) at a large-scale commercial dairy farm in
99 New South Wales, Australia between June and October 2018. The experiment was approved by the
100 University of Sydney Animal Ethics Committee (Project number: 2016/1078). Nineteen primiparous and
101 multiparous Holstein-Friesian cows were included in this experiment (n = 10 primiparous, n = 9
102 multiparous, mean parity = 2.16, range = 1 - 5). These experimental cows were selected from a herd of
103 maternity cows (n = ~ 120) which were all within two weeks of their intended date of parturition. From
104 this herd, each focal cow was selected on her day of parturition, based on her expression of prepartum
105 behaviour including increased vocal rate, restlessness, tail lifting, increased mucous production, looking
106 at other calves and/or licking her amniotic fluid (Barrier et al., 2012). On this farm, the calving
107 intervention criteria involved the farm staff checking the progress of the parturient cow every 30
108 minutes (min) (including pushing, visibility of discharge, amniotic sac and/or feet). Farm staff assisted
109 the cow when no progress was made for a minimum of 30 min. To minimise variability in calving
110 procedures, only cows that required calving intervention for foetal expulsion were included in this study.
111 Both parturition and subsequent calf separation were observed for all experimental cows from 06:30 –

112 14:30, with one cow recorded at a time. All cows were identifiable by their unique coat markings, ear
113 tags and collar numbers. Given their daily handling, all cows were habituated to human presence.

114



115

116 Figure 1. Diagram of the maternity barn, containing Pen 1 (11.7 m x 57.0 m), the pen with the cattle
117 crush (11.7 m x 2.7 m), Pen 2 (11.7 m x 9.2 m) and Pen 3 (11.7 m x 15.8 m), which are divided by a fence-
118 line as seen in (Green et al., 2020, under review).

119

120 2.2. Housing and feeding

121 Pre-parturient cattle were introduced to the maternity barn once a week. Cattle were grouped by parity,
122 with multiparous cows housed in a free-stall section (Pen 1) of up to 65 animals, and primiparous cows
123 loosely housed in Pen 3 with up to 12 animals (Figure 1). The multiparous cows had access to 68 (34 x 2)
124 free stalls which contained soft sand bedding that was raked daily. The majority of Pen 2 and Pen 3 were
125 covered in soft sand bedding which was replaced weekly. The remainder of the barn had grated
126 concrete flooring which was flushed daily. In their respective pens, all cows were offered a total-mixed
127 ration diet *ad-libitum* at a feeding strip on the far side of the barn. All cows had unlimited access to
128 water in troughs positioned throughout the maternity barn. At 07:00 and 15:00, cows that had calved
129 that day were processed for milking and moved to a separate milking barn and herd.

130

131 **2.3. Recording conditions**

132 *2.3.1. Parturition*

133 The recording of parturition commenced when the cow expressed signs of the second stage of labour,
134 including visibility of the amniotic sac and/or calf legs. Duration of recording varied between cows with
135 calving duration longer for primiparous (mean = 89.9 min, range = 50 – 173 min) than multiparous
136 (mean = 63.2 min, range = 24 – 127 min) cows. Despite the group-housing environment, during
137 parturition, cows tended to isolate themselves from conspecifics by moving to a pen area free from
138 disturbance. Calving intervention was provided by the experienced farm staff and involved using a
139 calving jack whilst the cow was standing restrained in a head bail at the cattle crush (n = 5 primiparous,
140 n = 8 multiparous cows) or calving ropes to pull the calf from the side lying cow (n = 5 primiparous, n = 1
141 multiparous cows). Recording ceased when the calf was born. During parturition, vocalisations were
142 mainly associated with the cow pushing out the calf, or when the farm staff assisted with calf pulling.
143 High-quality recordings were available for 17 of the 19 experimental cows during parturition.

144 *2.3.2. Calf separation*

145 Calf separation involved the farmer moving the calf to the other side of a fence-line barrier between Pen
146 2 and Pen 3 (Figure 1) where the calves and cows were housed, respectively. The focal cow was
147 recorded for 60 min immediately after separation from her calf, with separation occurring soon after
148 parturition (mean time = 108 min, range = 64 – 271 min). All cows could bond with their respective calf
149 before separation commenced. Across the observation period, recording coincided with peak calving
150 activity, meaning that Pen 2 contained multiple calves (mean = 5, range = 1 – 10). Calves had access to a
151 heated shelter to maintain body temperature if needed. Pen 3 consisted of multiple cows including the
152 post-partum focal cow and primiparous cows which were due to calve (mean = 7 cows, range = 4 – 10).

153 The focal cow and her respective calf maintained visual, auditory and some physical contact through the
154 fence-line barrier for the recording session. During calf separation, vocalisations were mainly produced
155 when the cow looked at the calf. Calves were transported twice daily at 10:00 and 15:00 h to a separate
156 shed for individual housing.

157

158 **2.4. Vocalisation recordings**

159 Vocalisations were recorded from the 19 cows across parturition and subsequent calf separation.

160 Vocalisations were captured from between 1 to 15 m from the focal cow using a Sennheiser ME67

161 directional microphone (frequency response: 40 – 20000 Hz, max SPL: 125 dB at 1000 Hz) powered by a

162 Sennheiser K6 power module (Sennheiser Electronic GmbH and Co, Wedemark, Germany). For shock

163 and wind-noise reduction, the microphone was covered with a Rycote Classic Softie Windshield and

164 attached to a Softie mount with pistol grip® (Rycote Microphone Windshields, Gloucestershire, United

165 Kingdom). Vocalisations were recorded onto a Marantz PMD661 MK2 digital solid-state recorder

166 (Marantz Professional, Cumberland, RI, United States) with stereo input (sampling rate: 44.1 kHz). Lower

167 and higher amplitude calls were captured using two recording volume gain settings concurrently and

168 these settings were adjusted manually to avoid signal saturation. Each vocalisation was stored as a

169 separate file in the .WAV uncompressed format at 16-bit amplitude resolution.

170

171 **2.5. Vocalisation analyses**

172 A total of 393 vocalisations were analysed on the basis of good signal to noise ratio, including 214 from

173 parturition (n = 9 multiparous cows, n = 8 primiparous cows) and 179 from calf separation (n = 7

174 multiparous cows, n = 5 primiparous cows). Vocalisations were analysed using Praat v.6.0.31 (Boersma

175 and Weenink, 2009). Overall, we measured 12 acoustic parameters (Table 1) that could be robustly
176 extracted from vocalisations produced in a commercial farming environment where ambient noise was
177 an important component of the soundscape. Further, we selected parameters that would likely
178 modulate with **increasing context-related urgency**. Full call duration was measured directly off the
179 waveform, and the narrow band spectrogram of each call was visualised (FFT method, window length =
180 0.1 s, time steps = 1000, frequency steps = 250, Gaussian window shape, dynamic range = 60 dB)
181 displaying the first 2500 Hz of the call, to determine the proportion of nonlinear phenomena relative to
182 the full call duration. We then ran a custom-built script to batch process the remaining acoustic analyses
183 (Reby and McComb, 2003), which contained two distinct procedures to characterise the F0 contour and
184 modulation events, respectively. In particular, a band-pass filter (pass Hann band, from frequency = 40
185 Hz, to frequency = 2500 Hz, smoothing = 10 Hz) was firstly applied to each vocalisation to remove
186 background noise produced by farm machinery and birds present in the maternity shed. The script
187 procedure then involved extracting the F0 contour of each vocalisation ([Sound: To pitch (cc) command],
188 time step = 0.01s, pitch floor = 45 Hz, pitch ceiling = 1000 Hz) to measure a range of F0-related
189 parameters. These included F0 mean, F0 min, F0 max, F0 start, F0 end, F0 var, F0 cv and F0 absolute
190 slope. We manually corrected spurious values and octave jumps by viewing the spectrogram and the
191 associated F0 contour using the Pitch edit window [Inspect pitch object] (Reby and McComb, 2003). In
192 the case of biphonation sidebands (Fitch et al., 2002), the F0 values were selected if they were clearly
193 visible. Further, we 'unvoiced' any regions where the F0 values could not be accurately detected, for
194 example, where deterministic chaos (Fitch et al., 2002) was present. The second procedure involved
195 applying two distinct smoothing algorithms to the F0 contour, including (Smooth command in Praat,
196 bandwidth = 25) followed by (Smooth command in Praat, bandwidth = 2), which suppressed the long-
197 term and short-term F0 modulation events, respectively (Koutseff et al., 2017; Raine et al., 2018; Reby
198 et al., 2016). Following the smoothing procedures, the inflection points were counted as a change in the

199 sign of the contour's derivative and divided by the total number of voiced segments in each recording.
 200 This resulted in two distinct indexes of F0 modulation, including inflex25, which characterised minor
 201 intonation events, and inflex2 which characterised major intonation events.

202

203 Table 1. Definitions and justification of the 12 vocal parameters measured in this study.

Parameter type	Vocal parameter	Definition	References to previous distress-related studies
Temporal	Full duration (s)	Total duration of the call (from start to end)	(Raine et al., 2018; Stoeger et al., 2012, 2011)
F0 - contour	F0 mean (Hz)	Mean F0 across the call	(Koutseff et al., 2017; Raine et al., 2019, 2018)
	F0 min (Hz)	Minimum F0 across the call	(Koutseff et al., 2017; Raine et al., 2019, 2018)
	F0 max (Hz)	Maximum F0 across the call	(Koutseff et al., 2017; Raine et al., 2019, 2018)
	F0 start (Hz)	F0 at the start of the call	(Koutseff et al., 2017)
	F0 end (Hz)	F0 at the end of the call	(Koutseff et al., 2017)
	F0 var (Hz/s)	Cumulative variation in the F0 contour in Hertz divided by call duration	(Briefer, 2012)
	F0 CV (Hz)	Coefficient of variation over the duration of the call	(Koutseff et al., 2017; Raine et al., 2019, 2018)
F0 - modulation	Abs F0 slope (Hz/s)	F0 mean absolute slope	(Briefer et al., 2019)
	Inflex 25	Index of minor F0 modulation	(Koutseff et al., 2017; Raine et al., 2019, 2018)
	Inflex 2	Index of major F0 modulation	(Koutseff et al., 2017; Raine et al., 2019, 2018)

Nonlinear phenomena	Nonlinear phenomena (%)	Proportion of the call that contains nonlinear phenomena including deterministic chaos (non-random broadband noise with no clear harmonic structure), biphonation sidebands (side frequencies which occur either side of the F0 and harmonics) and/or subharmonics (spectral components that are integer fractions of the F0)	(Koutseff et al., 2017; Raine et al., 2018; Stoeger et al., 2012)
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204

205 **2.6. Behavioural observations**

206 A video camera (Sony HDR-AS300 action cam®, Sony Australia Limited, North Sydney, Australia) was
 207 affixed along the fence-line to continuously record the focal cow behaviour during parturition and calf
 208 separation. To relate behaviour with vocal production, video and audio were simultaneously recorded
 209 and time stamped. To characterise the accompanying mouth and tongue positions of the cows during
 210 vocal production, behavioural analysis software BORIS v.7.7.4 (Friard and Gamba, 2016) was used.
 211 Mouth position upon vocalisation was recorded as either 1) closed, 2) mixed (including both closed and
 212 open mouth components), or 3) open. The proportion of the call duration for which the mouth was
 213 open was quantified and tongue exposure (tongue protruding and/or dorsally flexed inside of the
 214 mouth) was additionally reported. Mouth position was recorded for all 393 vocalisations; however,
 215 tongue position was only clearly visible in 295 of the vocalisations.

216

217 **2.7. Statistical analyses**

218 Statistical analyses were performed using R Studio v.1.2.1 335 (RStudio Team, 2020), an integrated
 219 development environment for R (R Core Team, 2020). For the vocal data, we used the ‘lme4’ package
 220 (Bates et al., 2015) to carry out linear-mixed effects models (LMMs) with each vocal parameter included
 221 as the response variable. These models included the fixed effects of context (parturition or calf
 222 separation) and parity (primiparous or multiparous), and their two-way interaction. Since cattle calls are

223 likely structured on a graded continuum (Kiley, 1972), the proportion of the call duration with the mouth
224 open was included as a covariate in the model, as mouth configuration can influence F0-related
225 parameters (Sebe et al., 2010). Additionally, to compensate for the variable duration of parturition
226 amongst cows, time duration of both contexts was normalised to range between 0 and 1 with the time
227 of each vocalisation included as a covariate. All fixed effects were checked for multicollinearity using the
228 'VIF function' (Fox and Weisberg, 2018), with all considered to be non-collinear as they resulted in
229 values of less than four. The random effect was cow ID to control for repeated measures from the same
230 individuals. Residuals were graphically inspected, and to approximate normal distribution and
231 homoscedasticity all vocal parameters bar *inflex2* and nonlinear phenomena were log transformed.

232 For the behavioural data, which included categorical response variables, we ran a series of generalised
233 linear mixed models (GLMMs) with binomial distribution in 'lme4', implementing the 'all versus one'
234 approach. The response was the behaviour of interest (closed, mixed, or open mouth) which was scored
235 as 1, versus the other two behaviours of that category scored as 0. Fixed effects included context
236 (parturition or calf separation) and parity (primiparous or multiparous) along with their two-way
237 interaction, and the covariate of normalised time. The random effect was cow ID to control for repeated
238 measures. As tongue exposure was produced exclusively during parturition, no further statistical tests
239 were applied to this behavioural parameter.

240 To assess the significance of the fixed effects in both the vocal and behavioural datasets, we compared a
241 full model including the fixed and random effects, to a null model, comprising the covariates and
242 random effect for which we were controlling. Any non-significant interaction terms were dropped from
243 the models, with models compared using Akaike's information criterion (AIC) (Akaike, 1974), with the
244 lowest scoring AIC model selected. Models were fitted with maximum likelihood estimation and
245 likelihood ratio tests were used to confirm the significance of the full models. We used Tukey adjusted

246 pairwise comparisons in the ‘emmeans’ package (Lenth et al., 2019) to compare results of parturition
247 and calf separation, and we report the estimated marginal means, along with their standard errors on
248 the response scale. The main effect of context is reported in instances where the context by parity
249 interaction was not significant. Further, we only report on fixed effects which were of relevance to our
250 hypothesis, i.e. we do not report on the values of the covariates. For all models, a probability < 0.05 was
251 considered statistically significant, and a statistical trend was considered at $0.05 < p < 0.1$.

252

253 **3. Results**

254 *3.1. Vocal parameters during parturition and calf separation*

255 All 12 vocal parameters measured in this study were significantly affected by context, or a context by
256 parity interaction (Table 2). Post-hoc comparisons of context showed that vocalisations produced during
257 parturition had a greater prevalence of nonlinear phenomena as well as a higher F0 min and F0 start
258 (Table 3). By contrast, vocalisations during calf separation had a higher F0 max, and a greater F0 var, F0
259 cv and F0 absolute slope (Table 3). Additionally, these calf separation calls tended to have a higher F0
260 end (Table 3). There was a significant context by parity interaction for four vocal parameters including
261 full duration, F0 mean, minor F0 (inflex 25) and major F0 modulations (inflex 2) (Table 2). Within the
262 parturition context, primiparous cows produced vocalisations with a longer duration, a lower F0 mean,
263 and more major F0 modulations (inflex 2) than their multiparous conspecifics (Table 4). Primiparous
264 parturition vocalisations also tended to have more minor F0 modulations (inflex 25). By contrast, there
265 was no parity difference observed for any vocal parameter during calf separation (Table 4). Overall, for
266 both primiparous and multiparous cows, parturition induced calls with a longer duration, lower F0 mean
267 and more minor inflections (inflex 25) than subsequent calf separation (Table 4). Further, primiparous

268 cattle vocalisations contained more major inflections (inflex 2) during parturition than in subsequent calf
 269 separation, however for multiparous cows, this difference was not significant (Table 4).

270

271 Table 2. Results of final model selection for LMMs and GLMMs using ANOVAs, comparing the full model
 272 (with the fixed effects, covariates, and random effect) to the null model (with only the covariates and
 273 random effect). Only the fixed effects relevant to our hypothesis are provided in the table and
 274 significant P-values are highlighted in bold.

Parameter		Effect	df	X ²	P-value
Vocalisations					
Temporal	Full duration (s)	Context * Parity	3	62.16	< 0.001
F0 - contour	F0 mean (Hz)	Context * Parity	3	37.16	< 0.001
	F0 min (Hz)	Context	2	9.77	0.008
	F0 max (Hz)	Context	2	28.57	< 0.001
	F0 start (Hz)	Context	2	14.74	0.001
	F0 end (Hz)	Context	2	8.55	0.01
	F0 var (Hz/s)	Context	2	43.33	< 0.001
	F0 CV (Hz)	Context	2	32.03	< 0.001
	Abs F0 slope (Hz/s)	Context	2	35.23	< 0.001
F0 - modulation	Inflex 25	Context * Parity	3	19.27	< 0.001
	Inflex 2	Context * Parity	3	8.10	0.04
Nonlinear phenomena	Nonlinear phenomena (%)	Context	2	31.03	< 0.001
Behaviours					

Mouth position	Closed mouth	Context	2	5.59	0.06
	Mixed	Context	2	26.97	< 0.001
	Open mouth	Context * Parity	3	50.90	< 0.001

275

276 Table 3. Estimated marginal means \pm SE of main effects along with their P-values derived from the
 277 Tukey's post-hoc pairwise comparisons. The LMMs included each vocal parameter as the response
 278 variable, the fixed effects of context and parity, the covariates of normalised time and proportion of the
 279 mouth open, and the random effect of cow ID. Significant P-values are highlighted in bold.

Vocal parameter	Parturition	Calf separation	t-ratio	P-value
F0 min (Hz)	75.80 \pm 2.38	70.60 \pm 2.42	2.18	0.03
F0 max (Hz)	167.00 \pm 9.52	218.00 \pm 13.32	-4.99	< 0.001
F0 start (Hz)	84.80 \pm 3.58	75.70 \pm 3.42	2.97	0.003
F0 end (Hz)	119.00 \pm 4.89	130.00 \pm 5.89	-1.88	0.06
F0 var (Hz/s)	99.20 \pm 8.19	168.30 \pm 15.19	-6.01	< 0.001
F0 cv	0.15 \pm 0.01	0.23 \pm 0.02	-5.12	< 0.001
F0 abs slope	118.00 \pm 9.22	185.00 \pm 15.92	-5.22	< 0.001
NLP (%)	33.10 \pm 4.39	16.70 \pm 4.56	5.61	< 0.001

280

281

282 Table 4. Estimated marginal means \pm SE of interaction effects along with their P-values derived from the
 283 Tukey's post-hoc pairwise comparisons. The LMMs included each vocal parameter as the response
 284 variable, the interaction between the fixed effects context and parity, the covariates of normalised time
 285 and proportion of the mouth open, and the random effect of cow ID. Letters denote significant
 286 differences ($P < 0.05$) between parities in the same context, and P-values are reported for significant
 287 differences at the contextual level (parturition versus calf separation). Significant P-values are
 288 highlighted in bold.

Vocal parameter	Parity	Parturition	Calf separation	Parturition versus Calf separation	
				t-ratio	P-value
Full duration (s)	Primiparous	2.08 \pm 0.14 ^A	1.18 \pm 0.11	5.75	< 0.001
	Multiparous	1.52 \pm 0.10 ^B	1.17 \pm 0.07	5.36	< 0.001
F0 mean (Hz)	Primiparous	115.00 \pm 7.84 ^A	177.00 \pm 15.13	-5.01	< 0.001
	Multiparous	144.00 \pm 9.43 ^B	165.00 \pm 10.82	-3.35	< 0.001
Inflex 25	Primiparous	12.85 \pm 1.34	7.24 \pm 1.02	3.71	< 0.001
	Multiparous	9.92 \pm 0.97	8.42 \pm 0.82	2.10	0.04
Inflex 2	Primiparous	1.06 \pm 0.06 ^A	0.81 \pm 0.10	2.15	0.04
	Multiparous	0.87 \pm 0.06 ^B	0.88 \pm 0.05	-0.14	0.89

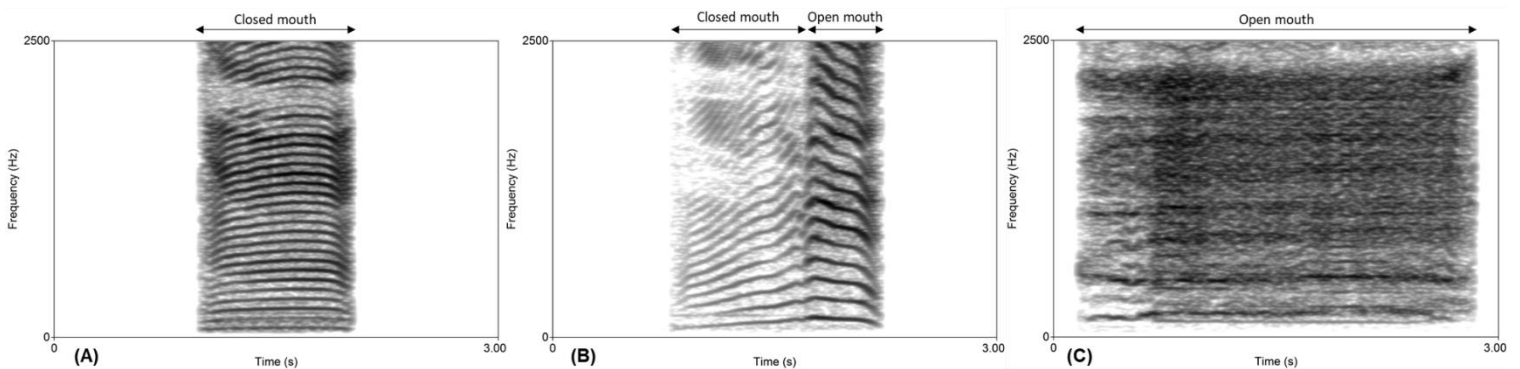
289

290

291 3.2. Phonatory behaviours during vocal production

292 The production of open mouth and mixed calls (Figure 2) was significantly impacted by context (Table 2).
293 Both open and mixed calls were emitted upon exhalation, and closed mouth calls were emitted nasally.
294 A greater percentage of mixed calls was observed during calf separation compared to parturition (Z ratio
295 = -4.73, $P < 0.0001$). By contrast, more open mouth calls were produced during parturition than calf
296 separation for both primiparous (Z ratio = 4.77, $P < 0.0001$) and multiparous cows (Z ratio = 3.02, $P =$
297 0.003). There was also a significant context x parity effect during parturition (Table 2), with primiparous
298 cows producing more open mouth calls than multiparous cows (Z ratio = -2.92, $P = 0.004$). During
299 parturition, tongue exposure was observed during 82% of the primiparous cow vocalisations and just
300 10% of the multiparous cow vocalisations, however this behaviour was not observed during calf
301 separation by any cow. Further, tongue exposure was exclusively observed when the cows were
302 emitting the vocalisation and at no other time during the observation period.

303



304

305 Figure 2. Narrow-band spectrograms of (A) a closed mouth vocalisation produced during parturition, (B)
306 a mixed vocalisation comprising closed and open mouth components produced during calf separation,
307 and (C) an open mouth vocalisation produced during parturition.

308

309 4. Discussion

310 Despite ongoing discussions about the potential for vocalisations to assess animal welfare (Green et al.,
311 2017; Manteuffel et al., 2004; Watts and Stookey, 2000), the information coded within cow vocalisations
312 remains under-investigated. Here we show context-related variability in the acoustic features and
313 phonatory behaviours of cow vocalisations recorded during parturition with dystocia and fence-line calf
314 separation. First, we describe the acoustic and phonatory-behavioural characteristics of peri-partum
315 cow vocalisations at the contextual level. We then discuss plausible reasons for their contextual
316 differences related to their communicative function and potential motivational and emotional encoding.
317 These findings contribute to the greater understanding of cattle vocal communication and are a step
318 towards the creation of a tool to detect welfare changes aurally and visually on farm.

319 Parturient cow vocalisations were characterised by a longer duration, overall lower F0, a greater
320 prevalence of nonlinear phenomena and more minor frequency modulations than calf separation calls.
321 Further, primiparous parturient cows produced calls longer in duration, more frequency modulated and
322 lower in F0 mean than their multiparous conspecifics. Presently, all cows had difficulty with foetal
323 expulsion, requiring farming intervention to extract the calf. Moreover, the primiparous cows endured
324 longer labour durations, which has previously been associated with more pain due to foeto-pelvic
325 disproportion and greater effort placed into calving (Mainau and Manteca, 2011). Interestingly, the
326 parturient cow vocalisations share features with the guttural, low F0 sounds of parturient women upon
327 effort and exertion (McKay and Roberts, 1990). We suggest that the parturient cow vocal characteristics
328 may reflect the intense musculature and energy expenditure during parturition, affecting sub-glottal
329 pressure and muscular tension within the vocal apparatus (Taylor and Reby, 2010; Titze, 1994). By
330 contrast, calf separation vocalisations could be discriminated by their distinctive F0 contour, being more
331 harmonic and comprising a broader frequency range and greater variability around the F0. It has been

332 shown that more intense separation methods (e.g. separation from the calf after four days) are
333 associated with calls of higher frequencies (Weary and Chua, 2000). Thus, the lack of parity effect on calf
334 separation call structure may represent within-context similarities in arousal expression. Our results also
335 align with the observations of Flower and Weary (2001), which found no behavioural differences
336 between post-partum primiparous and multiparous cows undergoing immediate separation from their
337 calves.

338 In our study, fundamental frequency related parameters (F0 mean, max, end, var, CV, absolute slope)
339 were overall higher for calf separation than parturition and did not conform to the vocal arousal
340 expression exhibited by other mammalian species (Briefer, 2012). In dogs, disturbance barks produced
341 in more urgent situations (e.g. when a stranger rang a doorbell) were also lower in F0 parameters,
342 despite mirroring higher arousal through their longer duration, more rapid repetitions and vocal
343 roughness (Yin and McCowan, 2004). Cattle vocalisations are acoustically graded (Kiley, 1972) and in our
344 study there was a high prevalence of intermediate call types which were ambiguous to visually classify.
345 For this reason, we addressed contextually driven vocal variation independent of any empirical call type.
346 Despite the ability to encode instantaneous dynamic information (Taylor and Reby, 2010), vocalisations
347 are not always direct expressions of emotional state. Different parts of the acoustic continuum may
348 serve different communicative functions in a variety of contexts, for example nasalised calls are often
349 favoured for short-distance communication, and oralised calls for communication over longer distances
350 (Padilla de la Torre et al., 2015). This could explain why it was difficult to compare the potential
351 emotional encoding in vocalisations across contexts. The emotional information encoding in other
352 mammalian species has been shown to differ between call types (Linhart et al., 2015; Maigrot et al.,
353 2018), thus to more definitively attribute acoustic variations to emotional state, future cattle studies
354 should analyse vocalisations most typical of the low- and high-frequency call structures (Padilla de la
355 Torre et al., 2015) and exclude any intermediate call sub-types.

356 Acoustic variations can reflect the circumstances surrounding vocal emission (Owings and Morton,
357 1998). Indeed, the present contrasting peri-partum acoustic features were most likely shaped by the
358 different communicative functions of the vocalisations. Low-frequency vocalisations tend to propagate
359 further than high-frequency vocalisations (Marten et al., 1977), with nonlinear phenomena enhancing
360 the projection of formant frequencies over longer distances (Fitch et al., 2002). Therefore, parturient
361 cows were likely vocalising over longer distances to their conspecifics, or to the farmer for help. In
362 contrast, when separated, the cows were likely vocalising to their respective calves in close proximity
363 across the fence-line. The calf separation calls emulate the high-frequency calls produced by free-
364 ranging cattle initiating social contact (Padilla de la Torre et al., 2015), similar in their acoustic structure
365 (F0 start, end, mean, min, max, absolute slope, var) and their mixed mouth configuration, suggesting
366 that these separation calls play a communicative role in maintaining contact with the calf. Nonetheless,
367 the acoustic characteristics of the peripartum vocalisations partially conform to Morton's motivational-
368 structural rules, which state that mammals emit harsh, low-frequency, high-intensity vocalisations when
369 in hostile contexts as opposed to tonal, high-frequency vocalisations when appeased or fearful (Morton,
370 1977). The communicative functions of these certain acoustic characteristics could be verified through
371 playback experiments of peri-partum vocalisations to signal receivers.

372 Just as acoustic properties can enhance the salience of the vocalisations for long distance
373 communication, seemingly so too can the phonatory behaviours associated with vocal production.
374 Parturient vocalisations were typically coupled with an open mouth configuration, and this was
375 observed more frequently in the primiparous than multiparous cows. Mouth configuration has been
376 used to empirically classify call types in cattle (Johnsen et al., 2015; Padilla de la Torre et al., 2015) and
377 other ungulates (Briefer et al., 2019, 2015b), with evidence to suggest that the expression of different
378 call types relates to different underlying motivational or emotional states (Maigrot et al., 2018; Morton,
379 1977; Tallet et al., 2013). A greater production of open mouth calls also seems to correlate with

380 increasing distress during calf separation (Johnsen et al., 2015), which could reflect greater urgency
381 during parturition compared to calf separation, especially for the primiparous cows.

382 Tongue protrusion was also observed exclusively during parturition solely at the time of vocal
383 production. To the best of our knowledge, this behaviour has not been documented in other cattle
384 studies, but in Iberian red deer stags, tongue protrusion is a conspicuous feature of acoustic rutting
385 display and is a consequence of thyroid muscle contraction (Frey et al., 2012). While hypothesised to be
386 energetically costly, tongue protrusion with an open mouth configuration may allow for the
387 uninterrupted passage of an exhalatory air stream producing the vocalisation (Frey et al., 2012), which
388 could mechanically function to project the vocalisation over longer distances. This could explain why
389 there was a greater prevalence of tongue protrusion behaviour in the primiparous parturient cows,
390 again reinforcing the notion that they perceived the context with greater urgency. Although we did not
391 directly estimate the impact of tongue protrusion on vocal characteristics, the lower F0 mean of the
392 parturient vocalisations, particularly those emitted by the primiparous cows, may have also been
393 influenced by the tongue protrusion behaviour. Similarly, the common roars of Iberian Red stags which
394 were produced with the tongue protruded, were lower in F0 than their non tongue-protruding harsh
395 roar equivalents (Frey et al., 2012). Presently, both mouth configuration and tongue protrusion
396 behaviour provided valuable information about contextual and parity-related effects in cows and are
397 promising visual tools to assess welfare-related differences on farm. Further exploration of these
398 phonatory behaviours and their impact on acoustic features is therefore warranted in a well-controlled
399 experimental setting to validate our current suggestions.

400 Our findings are subject to some limitations. Due to the observational design of the present study, the
401 two peripartum contexts differed in a number of aspects. The activity of the cow was higher during
402 parturition, as cows expended strong muscular efforts to expel the calf. Also, the position of the cows

403 upon vocalising differed, with parturient cows either recumbent or restrained in a head-bail, compared
404 to separated cows which were moving freely. This prevented the assessment of locomotion as a
405 behavioural proxy of arousal (Leliveld et al., 2017; Maigrot et al., 2018), or heart rate as a physiological
406 indicator of arousal (Briefer et al., 2015b, 2015a), as there was no previous habituation to the monitors.
407 To overcome these limitations to unequivocally attribute acoustic differences to variations in emotional
408 state, a controlled experiment should be performed in future. This could also involve comparing
409 additional variables within each context, such as different methods of parturition (assisted versus
410 unassisted) as well as different times (Flower and Weary, 2001; Lidfors, 1996; Weary and Chua, 2000) or
411 degrees (Johnsen et al., 2015) of calf separation. By comparing calls with the same communicative
412 function, this would assist in deciphering the precise vocal indicators of emotion in peri-partum cow
413 vocalisations.

414

415 **5. Conclusions**

416 Vocalisations and associated phonatory behaviours can provide insight into the peri-partum farming
417 context to which cows are exposed. In this study, we identified a series of robust vocal parameters that
418 are not influenced by ambient noises produced on commercial dairy farms, and behavioural parameters
419 that are conspicuous and easy to observe. The combination of an open mouth configuration, tongue
420 protrusion and spectral properties such as an overall lower F0 and longer duration during parturition
421 likely resulted in signal propagation over longer distances, thereby informing more receivers about the
422 urgency of this context. We recommend assessing these measures further in a controlled experimental
423 setting, to simultaneously determine behavioural and vocal encoding of emotions in peri-partum cows,
424 for a more comprehensive understanding of their welfare state.

425

426 **Acknowledgements**

427 We are grateful to the undergraduate students who assisted with the data collection and farm staff who
428 were so accommodating to this research. Special thanks to Peter Thomson for the statistical advice and
429 Lena Lidfors for her behavioural expertise. Also, thank you to Frédéric Sèbe for assistance with filtering
430 the vocalisations.

431

432 **Funding**

433 Author A.G was supported by an Australian Government Research Training Program (RTP) Scholarship.
434 This research was partially supported by Dairy Australia to C.C and S.L and the University of Lyon's
435 IDEXLYON project as part of the "Programme Investissements d'Avenir" (ANR-16-IDEX-0005) to D.R.

436

437 **Author contributions**

438 A.G, C.C, S.L designed the experiment. C.C and S.L sourced funding and provided resources and project
439 administration. A.G collected the data. A.G, L.F, D.R conducted the acoustical and statistical analyses.
440 A.G, L.F, D.R conceptualised the manuscript. A.G wrote the first draft of the manuscript. C.C, S.L, L.F, D.R
441 contributed to subsequent drafts of the manuscript.

442

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