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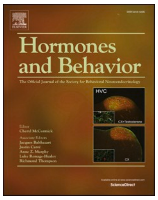

Acoustic correlates of physiological stress in a wild primate

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ABSTRACT

Vocalizations potentially encode information about physiological states, yet there is little direct evidence linking vocal parameters to physiological stress in non-humans, including primates. We investigated whether male mantled howler monkey (*Alouatta palliata*) loud calls reflect physiological stress by analyzing the relationships between the acoustic parameters of loud calls and fecal glucocorticoid metabolite (fGCM) concentrations. Howler monkeys produce loud calls primarily in the context of intergroup competition, which has the potential to elicit physiological stress responses. We expected that elevated fGCM would be associated with loud call acoustics through changes in laryngeal tension (increasing fundamental frequency and vocal perturbations), respiratory control (affecting call duration and temporal patterning), and vocal tract configuration (modifying spectral properties). We analyzed 93 high-quality loud calls and assayed 242 fecal samples collected over a 10-year period from 23 adult males across seven groups in Los Tuxtlas (Mexico). We calculated 26 loud call acoustic measurements including spectral, temporal, and non-linear variables. Mixed-effects modeling revealed that acoustic features collectively explained 71 % of the variation in fGCM. Loud calls produced at higher fGCM were characterized by increased pitch, greater pitch instability, altered vocal tract resonances, increased voice roughness, and reduced tonal clarity. Among these features, changes in pitch showed the strongest association with fGCM. These findings establish a link between stress physiology and vocal production in howler monkeys, suggesting that internal physiological states manifest in acoustic signals that could convey information about caller condition.

1. Introduction

Acoustic communication plays a critical role in the evolution, ecology, and behavior of animals, serving as a primary means for conveying information across diverse contexts, including mating, territory defense, predator avoidance, and social coordination (Bradbury and Vehrencamp, 2011; Catchpole and Slater, 2008; Wiley and Richards, 1978). Vocal signals are particularly advantageous as they can travel over long distances, bypass visual obstructions, and be produced in various environmental conditions (Gerhardt and Huber, 2002; Slabbekoorn and Smith, 2002). Understanding what information is encoded in vocalizations is essential for interpreting how animals interact with con- and hetero-specifics in different environments (Fitch and Hauser, 2002; Owren and Rendall, 2003; Seyfarth and Cheney, 2003). Vocal signals often convey information about the identity, emotional state, physical condition, and social status of the caller (Briefer, 2012; Charlton et al., 2007; Ey et al., 2007; Taylor and Reby, 2010). Consequently, understanding the form and function of acoustic signals can provide insights into the evolutionary pressures shaping communication systems and reveal critical aspects of an animal's ecology and social dynamics (Endler, 1992; Freeberg et al., 2012; McGregor, 2005; Owren and Rendall, 2003).

The physiological state of an individual can significantly influence the acoustic properties of its vocalizations, often serving as honest indicators of internal states such as experiencing an active stress response, reproductive condition, and health (Briefer, 2012; Ey et al., 2007; Koren et al., 2008; Nowicki and Searcy, 2004). Hormonal modulation, particularly through glucocorticoids, can play a pivotal role in this

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process (Scherer, 2009). Glucocorticoid hormones, released in response to various stimuli, have widespread effects on muscle systems throughout the body by binding to receptors that alter muscle contractility, tone, and elasticity (Sapolsky et al., 2000). In the context of vocalization production, these general muscular effects manifest specifically in the laryngeal apparatus, where glucocorticoids modulate the tension and elasticity of intrinsic laryngeal muscles (Jürgens, 2009; Mukudai et al., 2015; Titze, 1994). This modulation directly impacts vocal fold vibration patterns, leading to alterations in pitch, amplitude, and call rate (Giddens et al., 2013; Hammerschmidt and Fischer, 2008; Riede and Goller, 2010; Titze and Abbott, 2012). Beyond the larynx, glucocorticoids also affect the respiratory muscles, altering breathing patterns and subglottal pressure (i.e., air pressure below the vocal folds that drives vocalization), which in turn influence the intensity and duration of vocalizations (Kikusui et al., 2011; Scherer, 2003). These combined effects result in specific acoustic signatures: elevated glucocorticoid levels in mammals have been associated with increased frequency modulation (jitter), amplitude perturbation (shimmer), and higher fundamental frequency (Blumstein and Chi, 2012; Briefer, 2012; Giddens et al., 2013; Taylor and Reby, 2010; Sehrsweeney et al., 2019; Viljoen et al., 2014). These acoustic parameters have shown particularly consistent relationships with physiological stress across diverse mammalian species, making them promising candidates for analysis in primates (Briefer, 2012; Linhart et al., 2015; Scherer, 2009). Thus, investigating the interaction between glucocorticoid function and the acoustics of vocalizations provides valuable insights into the mechanisms underlying animal communication and the ecological relevance of vocal signals (Briefer, 2012; Ey et al., 2007).

Howler monkeys (genus *Alouatta*) produce distinctive roar vocalizations (sometimes called howls) characterized by long duration and low fundamental frequencies, generated through their highly specialized vocal apparatus that includes an enlarged hyoid bone acting as a resonating chamber (Dunn et al., 2015; Schön, 1964, 1971). Howler monkey loud call bouts are variable in terms of duration and composition, usually including several occurrences of roars, barks, insipient roars, and insipient barks (Baldwin and Baldwin, 1976; da Cunha et al., 2015). Roars are the vocalization type with highest energy, and a loud call bout usually includes one to four roar respiratory cycles, with each cycle consisting of one exhaled and one inhaled syllable (Baldwin and Baldwin, 1976; Fig. 1). These powerful, low-frequency roars can travel over 1 km through dense forests, an essential adaptation for communication in the diverse Neotropical habitats they occupy from Mexico to Argentina, including rainforests and dry forests where visibility is limited (da Cunha et al., 2015; Di Fiore and Campbell, 2007).

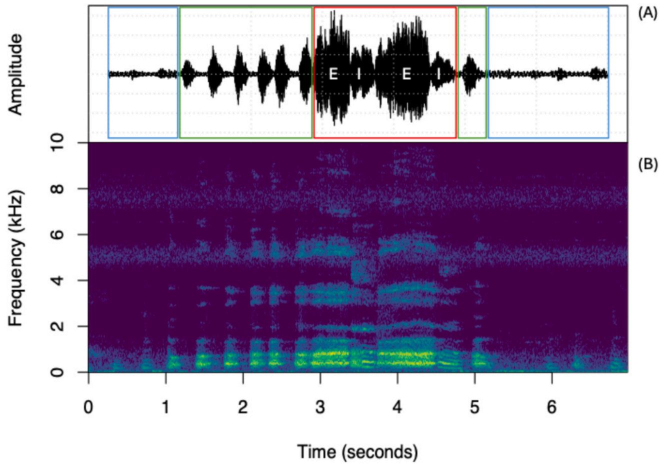

Fig. 1. A typical mantled howler monkey loud call. The different components of the call can be visualized in the oscillogram (A): it starts with two insipient barks (first blue rectangle), followed by six barks (first green rectangle), then by four roar syllables (red rectangle), one bark (second green rectangle), and two insipient barks (second blue rectangle). The roar component of the call is composed of two cycles, each including one exhalation (E) and one inhalation (I). The strongest energy is associated with roars (B) and occurs between 2.5 and 5 s. In the present study we focused on the first cycle of roars.

Roars serve critical social functions, being used primarily for intergroup spacing, territorial defense, and mate attraction, allowing groups to communicate their location and maintain spatial relationships without direct visual or physical contact (da Cunha et al., 2015; Kitchen et al., 2015). The acoustic structure of these roars varies predictably with caller characteristics including age, sex, and social context (da Cunha et al., 2015). Several factors influence the production and use of these loud calls, with vocal behavior varying according to group composition, reproductive status, resource availability, and anthropogenic disturbances (Kitchen et al., 2015). However, despite extensive research on the social and ecological factors affecting howler monkey vocalizations, we currently lack data on how or whether internal states, particularly physiological stress, contribute to acoustic variation in these loud calls.

A widely used measure of internal state in vertebrates is circulating glucocorticoid levels. Glucocorticoids are steroid hormones produced by the adrenal cortex that play crucial roles in metabolism, immune function, and physiological stress responses (Sapolsky et al., 2000). Glucocorticoids are secreted in response to physical and psychological challenges and in mantled howler monkeys (*A. palliata*), elevated fecal glucocorticoid metabolite concentrations (fGCM) have been linked to resource scarcity, intergroup encounters, and human-induced disturbances (e.g., Cañadas-Santiago et al., 2020; Cristóbal-Azkarate et al., 2007; Dias et al., 2017, 2022; Rangel-Negrín, 2023; Dunn et al., 2013). Although some studies report concurrent variation in vocalizations and glucocorticoids (Cañadas-Santiago et al., 2020; Gómez-Espinosa et al., 2022; Rangel-Negrín et al., 2023), direct evidence of glucocorticoid-linked modulation of acoustic parameters is lacking in this species. Addressing this knowledge gap is critical for advancing our understanding of the ecological and social significance of vocal signals. From a behavioral ecology perspective, if vocalizations reliably encode information about an individual's physiological state, this could influence social interactions, mate choice, and conflict dynamics (Bercovitch and Ziegler, 2002; Charlton et al., 2007; Creel et al., 2013; Wich and Nunn, 2002). From a conservation standpoint, non-invasive acoustic monitoring could serve as a cost-effective alternative to physiological sampling, offering valuable tools for assessing animal welfare and habitat quality in wild populations (Blumstein et al., 2011; Kalan et al., 2015).

We examined the relationship between physiological stress and the acoustic properties of roar vocalizations in mantled howler monkeys. We hypothesized that elevated physiological stress may alter vocal production through three primary physiological mechanisms:

(1) Glucocorticoids can bind to receptors in laryngeal muscles, altering their contractile properties (Titze, 1994; Sapolsky et al., 2000), and stress-induced changes in vocal fold dynamics directly influence frequency and perturbation parameters (Briefer, 2012; Giddens et al., 2013; Herbst et al., 2025). By increasing tension in laryngeal muscles, these changes should influence source-related parameters: mean f0, f0 range, f0 standard deviation, local jitter, RAP jitter, local shimmer, APQ shimmer, and zero crossing rate (Tables 1 & S1).

(2) Stress hormones influence respiratory muscle function and neural control of breathing, altering subglottal pressure and respiratory drive during vocalization (Scherer, 2003; Titze and Abbott, 2012). Therefore, they may influence temporal and energetic features of calls: call duration, temporal centroid, spectral flatness, and peak frequency (Table 1).

(3) Stress-induced muscle tension may extend to the supralaryngeal apparatus, modifying vocal tract shape and resonance properties (Fitch and Hauser, 2002; Scherer, 2009; Taylor and Reby, 2010). Changes in vocal tract configuration, should impact filter-related parameters: formants (F1-F5), formant dispersion, mean formant spacing, formant stability, coefficient stability, spectral tilt, spectral centroid, mean dominant frequency, entropy, and frequency jumps (Table 1).

Table 1
Variables pertaining to the roars of mantled howler monkeys analyzed in this study.

Parameter	Description and biological significance
Mean f0 (Hz)	Average fundamental frequency. Reflects primary rate of vocal fold vibration and correlates with body size and hormonal status.
f0 Range (Hz)	Range between minimum and maximum fundamental frequencies. Indicates vocal flexibility and potential emotional state.
f0 standard deviation (Hz)	Variation in fundamental frequency. Shows degree of frequency modulation and pitch stability.
Local jitter	Measures the cycle-to-cycle variation in the fundamental frequency of a vocalization.
Relative average perturbation (RAP)	RAP jitter is a more smoothed measure of frequency perturbation that considers three periods at a time
Local shimmer	Local shimmer measures the cycle-to-cycle variation in the amplitude of a vocalization
Amplitude perturbation quotient (APQ)	APQ shimmer is a smoothed measure of amplitude perturbation that considers five periods at a time.
Zero crossing rate (crossings/s)	Rate of signal polarity changes. Indicates frequency content and signal periodicity.
Duration (seconds)	Total length of the vocalization. Indicates temporal extent of vocal production effort and energetic investment.
Temporal centroid (seconds)	Center of gravity of signal's energy distribution over time. Shows temporal organization of vocal energy.
Spectral flatness (ratio)	Geometric mean of power spectrum divided by arithmetic mean. Indicates tonality vs noise.
Peak frequency (Hz)	Frequency with highest amplitude. Represents most energetic component of vocalization.
Formant 1 (Hz)	First formant frequency. Reflects first resonance of vocal tract usually correlating with tongue height.
Formant 2 (Hz)	Second formant frequency. Related to front-back tongue position and vocal tract constriction.
Formant 3 (Hz)	Third formant frequency. Provides additional information about vocal tract shape and articulation.
Formant4 (Hz)	Fourth formant frequency. Further characterizes vocal tract resonances and articulation.
Formant5 (Hz)	Fifth formant frequency. Highest tracked formant providing detailed vocal tract information.
Formant dispersion (Hz)	Spacing between formants. Provides information about vocal tract size and shape.
Mean formant spacing (Hz)	Average distance between adjacent formants. Used to estimate vocal tract length.
Formant stability	Consistency of formant frequencies over time. Indicates articulatory precision.
Coefficient stability	Stability of LPC coefficients. Shows consistency in vocal tract configuration.
Spectral tilt	Rate of amplitude decrease across frequency. Indicates vocal effort and voice quality.
Spectral centroid (Hz)	Weighted mean frequency of spectrum. Correlates with perceived brightness and vocal effort.
Mean dominant frequency (Hz)	Average of most prominent frequency throughout vocalization. Indicates most energetic frequency component.
Entropy (bits)	Measure of spectral disorder. Higher values indicate more noise-like signals; lower values indicate more tonal sounds.
Frequency jumps (count)	Number of significant frequency transitions. Shows complexity of frequency modulation.

By examining this comprehensive set of acoustic parameters, we aimed to identify the specific vocal signatures of physiological stress in howler monkey vocalizations and determine which aspects of vocal production are most related to glucocorticoid elevation.

2. Methods

2.1. Ethical note

Our study was noninvasive and adhered to the American Society of Primatologists Code of Best Practices in Field Primatology and Principles for the Ethical Treatment of Nonhuman Primates. Research protocols were approved by the Secretaría de Medio Ambiente y Recursos Naturales of Mexico (permits SGPA/DGVS/10637/11, SGPA/DGVS/04999/14, SGPA/ DGVS/0381/17, SGPA/DGVS/13528/19, SGPA/DGVS/04015/21, and SPARN/DGVS/00278/22) and complied with the legal requirements of the Mexican law (NOM-059-SEMARNAT-2010).

2.2. Study sites and subjects

We conducted the study at the Los Tuxtlas Biosphere Reserve, in Veracruz, Mexico. The vegetation in this area was originally tropical evergreen rainforest but has been highly fragmented due to human activity (Dirzo and García, 1992). Mantled howler monkeys live in forest fragments throughout the area (Alcocer-Rodríguez et al., 2021; Cristóbal-Azkarate et al., 2017). We focused on 23 adult males (Table 2) that we could easily identify through natural markings, such as patches of yellow fur in their tails and feet, scars, and facial features.

2.3. Recording and processing vocalizations

We conducted fieldwork between January 2012 and December 2021 (Table 1). During visits, we recorded naturally occurring male loud calls ad libitum with a Sennheiser MKE600 directional microphone and a Marantz PMD660 digital recorder. Each time a call was recorded we noted the time of the day, and the stimulus associated with call production as anthropogenic (when the call was preceded by human-made noise or human presence), intergroup (when the call was preceded by a call from another group), or spontaneous (when no stimulus seemed to precede the call).

Following previous research, we defined a loud call bout as a period of loud calling separated from another one by at least 10 min (Bergman et al., 2016; Hopkins, 2013). We recorded a total of 1725 loud call bouts, of which 82 % corresponded to bouts in which two or more males were involved. We focused on loud calls produced by a single male ($n = 310$)

Table 2
Study sites, groups, subjects, and sampling effort.

Location	Group [a]	Male ID	No. calls	No. fGCM measurements
18°26.519′ N, 95°2.905 W	Group 1	1	3	9
		2	2	4
		6	3	8
		7	7	22
		8	3	8
		9	5	13
18°26.363′ N, 95°3.165 W	Group 2	10	4	11
		11	5	15
		12	4	15
		13	7	15
		14	3	9
18°26.768′ N, 95°2.944 W	Group 3	16	8	18
		17	4	14
18°37.110′ N, 95°5.128 W	Group 4	22	7	20
		23	4	8
		24	4	9
18°38.477′ N, 95°5.460 W	Group 5	30	3	5
		31	2	4
		34	2	6
		35	2	6
		37	5	11
18°36.440′ N, 95°4.225 W	Group 6	39	3	12
		40	3	9
Total		*23*	*93*	*242*

Sampling schedule: Groups 1 and 2 January 2012 to December 2021; Group 3 March to October 2019; Groups 4 and 5 January to October 2017; Group 6 February to August 2020.

to be able to match call acoustic attributes and fecal glucocorticoid metabolite concentrations at the individual level (see next section), from which we selected 252 high-quality calls (i.e., no clipping, background noise, or interruption by other callers). We used Audacity 3.7.1 (Audacity Team, 2024) to digitize loud call recordings and isolate individual roars as wav audio files (44.1 kHz sample rate, 16-bit resolution, mono format). To ensure consistent comparison across analyzed roars, we specifically isolated the first roar cycle of each loud call recording.

2.4. Glucocorticoid measurements

Mantled howler monkeys display a peak in fGCM concentrations approximately 20 to 30 h after experiencing an acute stressor, such as capture, anesthesia, or handling (Aguilar-Cucurachi et al., 2010; Cañadas-Santiago et al., 2020). Based on this knowledge, we determined fGCM concentrations in all fecal samples that could be matched to a recorded roar during the previous two days ($N = 347$).

Fecal samples were collected directly from the forest floor immediately after defecation when individual male identity could be confirmed without ambiguity. Each sample was placed in a polyethylene bag labeled with the individual's identity and temporarily stored in a cooler with ice packs during fieldwork before being transferred to a freezer maintained at −20 °C at the field station. All fecal samples underwent freeze-drying (FreeZone 18, Labconco, Kansas City, MO) within 6 months of collection. We extracted fGCM from these dried samples using a modified version of the Wasser et al. (2000) method. The process involved shaking 0.6 g of homogenized, lyophilized, and pulverized feces in 4.0-ml of analytical-grade methanol for 20 h. After centrifuging the extracts (460 g for 30 min), we collected the supernatant. Following complete solvent evaporation in a 60 °C water bath for 20 h, we reconstituted the resulting pellets with 3-ml albumin buffer for fGCM analysis.

fGCM concentrations were determined using a chemiluminescent immunoassay with a commercial kit (Cortisol, Immulite, Siemens, Los Angeles, CA, USA; sensitivity = 5.5 nmol/L; calibration range = 28–1380 nmol/L) and an automated immunoassay system (Immulite 1000 analyzer, Siemens, Munich, Germany). The antibody used in this kit is highly specific for cortisol, with minimal (8.6 %) cross-reactivity with corticosterone. Fecal samples were analyzed in 11 different assays during the 2012–2021 study period. Assay accuracy ranged between R^2 0.93 and 0.98 ($P < 0.001$ in all tests; $n = 5$ to 8 samples), parallelism (t) ranged from 0.9 to 1.3 ($P > 0.05$; n = 5 sampled for all tests), and precision averaged between 8.2 and 12.1 % ($n = 4$ samples in all tests) for intra-assay controls and between 7.5 and 13.7 % for inter-assay controls (n = 4 samples tests). fGCM values are reported as ng/g (dry feces).

2.5. Data organization and analysis

We used a subset of roars ($n = 93$ audios) for which at least two fGCM concentrations were available ($n = 242$ fecal samples, mean ± SD = 11 ± 5.1 samples per male, 2.7 ± 0.7 samples per male per roar) to account for variability in diverse factors know to influence glucocorticoid function and fGCM excretion (Goymann, 2012; Palme, 2019).

In the analysis of the acoustic parameters of calls we focused on a broad set of variables related to vocal production, several of which have consistently been related to emotional state/arousal as well as those that are usually reported by howler monkey bioacoustics research (e.g., Bergman et al., 2016; da Cunha et al., 2015; Sehrsweeney et al., 2019; Viljoen et al., 2014). Our acoustic analysis of howler monkey roars employed three complementary approaches implemented in R: general acoustic analysis, Linear Predictive Coding (LPC) analysis, and non-linear acoustic analysis. We utilized specialized packages for bioacoustics research (tuneR, seewave) and signal processing (signal, nonlinearTseries, pracma). Audio files were loaded using `readWave` (tuneR) and converted to mono when necessary, using `mono` (tuneR). Signal preprocessing included detrending with `detrend` (pracma) and various filtering operations using `filtfilt` (signal) and `butter` (signal). All analyses maintained the original sampling rate of the recordings throughout processing.

For the general acoustic analysis, we examined spectral and temporal parameters using Fourier transformations. The fundamental frequency (f0) parameters were extracted using a self-developed function with a specialized "howler monkey" preset (f0 range 80–150 Hz: Bergman et al., 2016; da Cunha et al., 2015; Supplementary methods). Howler monkey roars are noisy, broadband sounds presumably generated via deterministic chaos, which challenges the measurement of f0 (Dunn et al., 2015). We therefore employed a consensus approach combining autocorrelation, cepstral, spectral, and zero-crossing methods with adaptive reliability scoring. This approach yielded mean f0, f0 range, f0 standard deviation, and peak frequency measurements. The zero-crossing rate was calculated as the rate of signal polarity changes. For temporal features, we measured call duration directly from the wave object using sample count divided by sampling rate. The temporal centroid was calculated as the center of gravity of the signal's energy distribution. We assessed acoustic perturbation using custom functions to measure both frequency modulation (jitter) and amplitude modulation (shimmer). Specifically, local jitter and Relative Average Perturbation (RAP) were calculated by measuring cycle-to-cycle variation in the fundamental frequency. Similarly, local shimmer and Amplitude Perturbation Quotient (APQ) were computed by quantifying cycle-to-cycle variation in amplitude. Spectral flatness was determined as the ratio of geometric to arithmetic mean of the power spectrum, indicating the degree of tonality versus noise.

The Linear Predictive Coding (LPC) analysis provided detailed information about formant structure. We performed a frame-by-frame approach with 4096-sample frames and 12th-order LPC models. For optimal formant detection, we employed a two-pass approach: first detecting formants F2-F6 with a bandpass filter using `ffilter` (seewave), then specialized detection of F1 using enhanced low-frequency processing. This yielded measurements of formants F1-F5, with each formant validated against literature-based reference values (F1: 358 Hz, F2: 712 Hz, F3: 1573 Hz, F4: 1916 Hz, F5: 2811 Hz; Bergman et al., 2016; da Cunha et al., 2015; Dunn et al., 2015). Formant dispersion was determined using multiple methods, primarily following Reby and McComb (2003), with alternative approaches (Johnson, 2020) when all formants were not available. Mean formant spacing was calculated as the average distance between adjacent formants. Temporal stability of the vocal tract configuration was assessed via LPC coefficient stability, calculated as the mean of standard deviations of LPC coefficients over time. Formant stability was measured as the mean of standard deviations of formant frequencies over time. Spectral tilt was calculated as the logarithmic ratio of higher to lower formant energies, representing the rate of amplitude decrease across frequencies.

For the non-linear acoustic analysis, we focused on characterizing signal complexity and non-linear phenomena. This included the calculation of spectral entropy as a measure of signal complexity. The spectral centroid was computed as the weighted mean frequency of the spectrum using `spectro` (seewave) for the initial spectral analysis. Mean dominant frequency was calculated by averaging the frequencies with maximum amplitude across time windows. Frequency jumps were identified using a threshold-based approach to detect rapid, non-linear transitions in the frequency structure (Herbst et al., 2025), counting significant frequency changes exceeding 20 % of the mean frequency. For this analysis, we employed smaller window sizes (256 samples) in the `spectro` function (seewave) to capture rapid temporal variations in the signal structure.

As an initial exploratory step to examine potential relationships and guide subsequent modeling, we first calculated Pearson correlation coefficients between fGCM concentrations and each acoustic parameter, acknowledging that this approach does not account for the repeated-measures structure of our data. Given the large number of tests conducted, we adjusted significance values with the Benjamini-Yekutieli false discovery rate correction.

Although our biological hypothesis posits that glucocorticoid hormones influence vocal parameters, we modeled fGCM as the response variable with acoustic parameters as predictors to avoid multiple univariate tests and maintain statistical efficiency. This analytical approach identifies the same associations between hormones and acoustics while allowing us to examine all parameters simultaneously in a single model framework, with the understanding that our results demonstrate correlational relationships rather than causal directionality. We assessed variation in fGCM as a function of acoustic parameters using mixed-effects models. Prior to modeling, we conducted a thorough collinearity analysis of the acoustic parameters, employing a stepwise approach to assess multicollinearity using Variance Inflation Factors (VIF). The procedure started by testing individual predictors' relationships with fGCS and progressively built more complex models while maintaining VIF values below 5, ensuring the stability and interpretability of the final model. This collinearity assessment was based solely on relationships among predictors themselves, not on their associations with fGCM, thus avoiding selection bias that could arise from outcome-based variable selection. The selected acoustic parameters were standardized by centering and scaling (subtracting the mean and dividing by the standard deviation) to facilitate comparison of effect sizes across different measurement scales. We then constructed a linear mixed-effects model with log-transformed fGCM concentrations as the response variable, the selected acoustic parameters as fixed predictors, and male identity as a random effect to account for repeated measurements from the same individuals.

We followed an information-theoretic approach and multimodel inference to generate a set of models based on all combinations of predictors and then averaging model parameters across all resulting models and calculating the relative importance of each predictor based on its Akaike weights across all possible models (Burnham and Anderson, 2013). The marginal and conditional R^2 values for the averaged model were computed as weighted averages of R^2 values from individual component models, with weights corresponding to each model's contribution to the model averaging. We also examined the influence of variables that have been described as potentially influential in the vocal behavior of mantled howler monkey males on the relationship between fGCM concentrations and acoustic parameters: group membership (e.g., Ceccarelli et al., 2021; Hopkins, 2013; Maya-Lastra et al., 2025), stimuli associated with call onset (Whitehead, 1987), and time of the day (Chivers, 1969; Whitehead, 1987). We compared models with and without these variables using likelihood ratio tests.

3. Results

Correlation analysis of all 26 acoustic parameters with fGCM concentrations revealed significant relationships for 13 parameters (Fig. 2; Table S2). Among these, roar duration, f0 range, entropy, f0 standard deviation, temporal centroid, and frequency jumps showed moderate positive correlations with fGCM ($0.4 < r < 0.7$), whereas mean f0, local shimmer, formant 5, formant 1, local jitter, and Rap jitter had weak ($0.2 < r < 0.4$), although significant, correlations with fGCM.

We identified nine acoustic parameters that independently contributed to fGCM prediction through our VIF-based selection procedure: mean f0, f0 standard deviation, formant dispersion, local shimmer, entropy, temporal centroid, formant 5, formant 1, and frequency jumps (Fig. S1). The mixed-effects model incorporating these predictors significantly outperformed a null model that included only male identity

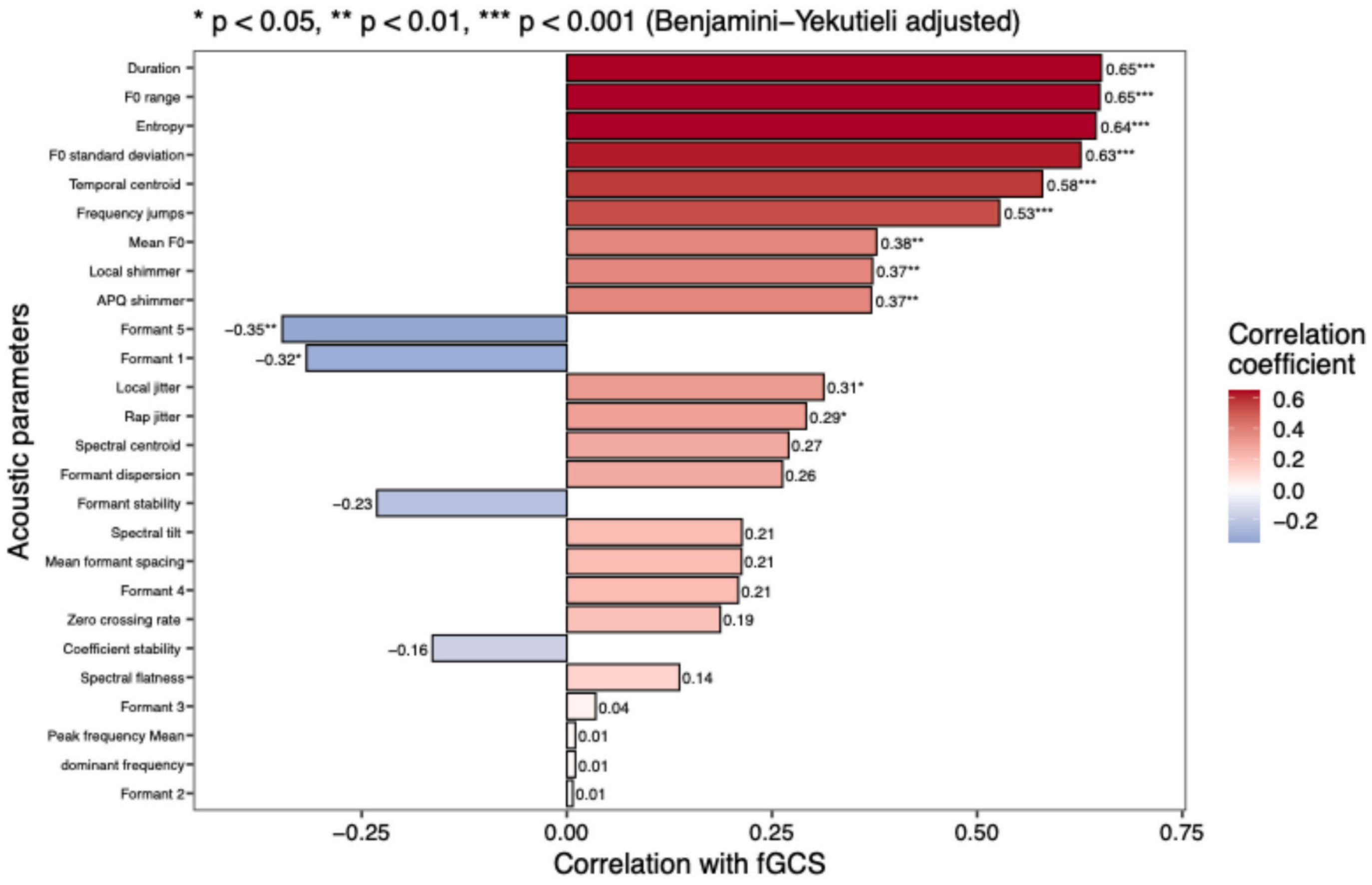

Fig. 2. Correlations (Pearson) between fecal glucocorticoid metabolite (fGCM) concentrations of mantled howler monkey males and the acoustic parameters of their roar vocalizations. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

as a random factor (LRT $\chi^2_6 = 130.3$, $P < 0.001$), and the small difference between marginal (0.71) and conditional (0.78) R^2 values (0.07) indicates that individual identity accounted for only ~9 % of the total variance in fGCM levels. Accordingly, within-individual variation (CV = 38.2 %) was substantially larger than between-individual variation (CV = 16.4 %). Therefore, fGCM variation was more strongly related to acoustic parameters than to individual differences among males (Fig. S2). The condition number for our selected acoustic parameters was 3.55, indicating no multicollinearity. The correlation matrix among predictors (Table S3) showed the highest correlation was $r = 0.68$ between frequency jumps and temporal centroid.

In the averaged model (Table 3), mean f0 demonstrated the strongest association with fGCM (estimate = 0.29) and had the highest importance among all parameters examined ($\sum W_i = 1.00$; Figs. 3 and 4). f0 standard deviation and formant dispersion also showed positive relationships with fGCM (estimate = 0.20) with high importance ($\sum W_i =$ 0.99), as did local shimmer (estimate = 0.17) and entropy (estimate = 0.21) with high importance ($\sum W_i$ = 0.97 and 0.92, respectively). Temporal centroid had moderate importance ($\sum W_i$ = 0.47) but no relationship with fGCM concentrations, as did formant 1, formant 5, and frequency jumps. Quantification of these relationships (after accounting for data transformations) indicates that a one standard deviation increase in mean f0 (15.62 Hz) was associated with a 33.7 % increase in fGCM (141.33 ng/g), corresponding to a 1.86 % increase per 1 Hz rise. For f0 standard deviation, a one standard deviation increase (6.81 Hz) resulted in a 22.3 % increase in fGCM (93.35 ng/g), equivalent to a 2.95 % increase per 1 Hz. Similarly, a one standard deviation increase in formant dispersion (39.42 Hz) was associated with a 21.6 % increase in fGCM (90.67 ng/g), representing a 0.50 % increase per 1 Hz. Local shimmer and entropy also showed substantial effects, with one standard deviation increases leading to 18.5 % (77.59 ng/g) and 23.4 % (98.12 ng/g) increases in fGCM levels, respectively.

Accounting for stimulus associated with call onset (LRT $\chi^2_2 = 3{,}1$, $P =$ 0.211) and time of the day (LRT $\chi^2_1 = 0.04$, $P = 0.857$) did not significantly improve the averaged model's performance in predicting fGCM, but group identity did (LRT $\chi^2_5 = 16.9$, $P = 0.005$).

4. Discussion

We aimed to examine whether the acoustic parameters of mantled howler monkey roars reflect physiological stress, as indicated by fecal glucocorticoid metabolite (fGCM) concentrations (although our statistical approach modeled these associations in reverse to efficiently examine multiple acoustic parameters simultaneously). Elevated physiological stress may alter vocal production through its effects on (i) laryngeal muscle function, (ii) respiratory muscle function and neural control of breathing, and (iii) muscle tension in the supralaryngeal apparatus. Our results revealed significant correlations between specific acoustic features and fGCM levels across all three domains, supporting the hypothesis that physiological stress influences vocal production through multiple physiological pathways.

Consistent with our prediction that stress influences laryngeal muscle tension, we found that source-related parameters (particularly mean f0, f0 range, f0 standard deviation, local jitter, and RAP jitter) showed significant correlations with fGCM levels. These findings suggest that increased physiological stress is associated with greater fundamental frequency values (i.e., the pitch of the voice, determined by how fast the vocal folds vibrate) and variability, as well as greater frequency and amplitude perturbations in howler monkey roars. This aligns with previous research indicating changes to the human voice under stress, with higher f0 and greater formant spacing (e.g., Pisanski and Sorokowski, 2020; Schrader and Todt, 1998; Tolkmitt and Scherer, 1986). It is also consistent with research on platyrrhines showing that different oscillatory states of the vocal apparatus produce distinct acoustic patterns, with laryngeal tension as a key determinant of fundamental frequency characteristics (Herbst et al., 2025). From a biological perspective, this is likely because glucocorticoid hormones modulate muscle tone and respiratory patterns (Sapolsky et al., 2000), thereby influencing vocal fold vibrations (Briefer, 2012; Riede and Goller, 2010). The physiological pathway likely involves glucocorticoids binding to receptors in laryngeal muscles, altering muscle tension and contractility (Jürgens, 2009), which directly affects vocal fold vibration patterns and creates the observed perturbations in frequency (jitter) and amplitude (shimmer).

In line with our prediction that stress influences respiratory control, we found significant correlations between fGCM and temporal parameters, particularly call duration and temporal centroid. These relationships may reflect stress-induced changes in respiration rate and depth that modify subglottal pressure, further affecting vocal production (Giddens et al., 2013; Titze, 1994). Glucocorticoids affect respiratory muscle function and neural control of breathing (Scherer, 2003; Titze and Abbott, 2012), leading to alterations in the temporal organization of vocalizations, as observed in our results.

Supporting our prediction about stress-induced changes in vocal tract configuration, we found significant relationships between fGCM and filter-related parameters, including formant measures (particularly formants 1 and 5), formant dispersion, spectral centroid, entropy, and frequency jumps. These associations reflect how stress-induced tension extends to the supralaryngeal structures (Fitch and Hauser, 2002; Scherer, 2009), altering vocal tract shape and resonance properties (Linhart et al., 2015).

The modeling approach revealed differences between simple correlation results and the final predictor selection in the mixed-effects model. While some acoustic parameters displayed strong correlations with fGCM (e.g., f0 range), they were excluded from the final model due to collinearity with other parameters. For example, some strongly correlated measures may have shared variance with mean f0, leading to the retention of just one of these measures in the final model to avoid multicollinearity. This statistical necessity reflects the biological reality that stress simultaneously affects multiple aspects of vocal production through shared physiological pathways. Interestingly, our averaged model included significant predictors from all three physiological mechanisms we predicted: source-related parameters (mean f0, f0 standard deviation, local shimmer), filter-related parameters (formant dispersion, formant 1, formant 5, entropy), and indicators of respiratory function (temporal centroid). These acoustic changes would manifest perceptually as roars with elevated pitch, greater pitch instability, increased harshness (perceived as voice roughness due to irregular vocal fold vibration), and reduced tonal clarity (higher spectral disorder). Collectively, these modifications would produce vocalizations that are acoustically distinct from those produced under lower stress conditions, potentially conveying information about the caller's physiological state to conspecific listeners. This highlights how physiological stress can affect multiple aspects of vocal production concurrently.

The mixed-effects modeling results showed that mean f0 had the

Table 3
Mixed effects model results on the relationship between fecal glucocorticoid metabolite (fGCM) concentrations and the acoustic parameters of the roar vocalizations of mantled howler monkey males.

Parameter	Estimate	SE	95 % CI		$\sum W_i$
			Lower	Upper	
(Intercept)	6.039	0.049	5.942	6.136	
Mean f0	0.290	0.043	0.206	0.375	1.0000
f0 standard deviation	0.201	0.040	0.123	0.279	0.9997
Formant dispersion	0.196	0.043	0.113	0.279	0.9996
Local shimmer	0.170	0.054	0.082	0.266	0.9733
Entropy	0.210	0.089	0.091	0.362	0.9281
Temporal centroid	0.066	0.083	0.018	0.266	0.4721
Formant 5	0.030	0.055	0.013	0.203	0.2803
Formant 1	0.003	0.017	−0.051	0.154	0.0583
Frequency jumps	0.002	0.015	−0.080	0.152	0.0496

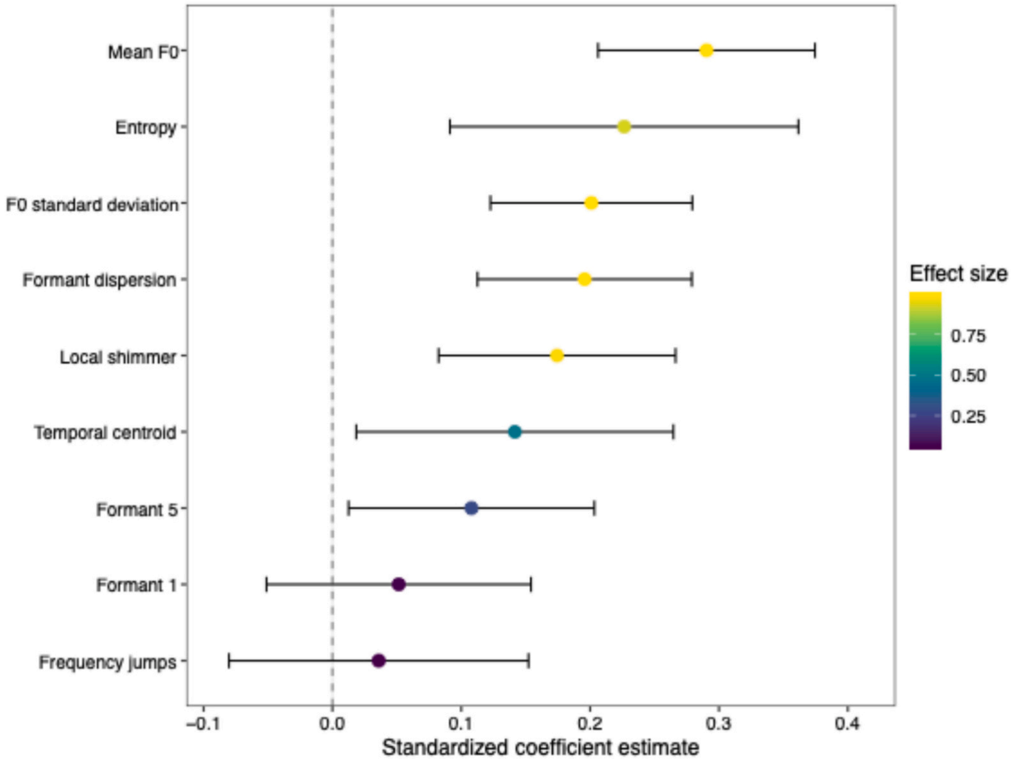

Fig. 3. Coefficient plot of the mixed-effects averaged model on the relationships between fecal glucocorticoid metabolite (fGCM) concentrations of mantled howler monkey males and selected acoustic parameters of their roar vocalizations.

strongest association with fGCM and had the highest importance among all parameters examined. This finding is particularly valuable for the field, as mean f0 is a well-established and extensively studied acoustic parameter in the literature (Titze, 1994; Taylor and Reby, 2010; Briefer, 2012), making these results more readily interpretable and applicable within the existing research framework. f0 standard deviation and formant dispersion also showed positive relationships with fGCM with high importance, as did local shimmer and entropy. This combination of parameters provides a biologically meaningful representation of how acoustic features encode physiological stress, capturing both source characteristics (f0 parameters), filter components (formant measures), and spectral properties (entropy). The model thus reinforces the proposition that vocal modulations reflect underlying hormonal influences on both the source (vocal folds) and filter (vocal tract) components of sound production (Fitch and Hauser, 2002; Hammerschmidt and Fischer, 2008; Kikusui et al., 2011; Owren and Rendall, 2003; Titze, 1994). Even if our modeling approach may have failed to capture complex, non-linear relationships between stress physiology and vocal production, the robust performance of the model indicates that acoustic parameters can provide meaningful insights into howler monkey physiological state, consistent with findings in other mammalian species (Briefer, 2012). It also supports the notion that vocalizations can serve as honest indicators of caller condition (Charlton et al., 2007; Nowicki and Searcy, 2004).

Several factors should be considered when interpreting the relationship between acoustic parameters and fGCMs. Biological factors such as volitional control over vocal production may influence how stress manifests in vocalizations, as primates demonstrate varying degrees of voluntary control over vocalizations within their repertoire (Coudé et al., 2011; Hage and Nieder, 2013). Howler monkeys may retain some ability to regulate aspects of their calls despite physiological constraints. Additionally, alternative non-causal explanations for the observed associations warrant consideration. While our data showed no effect of stimulus type on the relationship between fGCM and roar acoustic parameters, we did not measure the intensity of stimuli. It remains possible that more intense intergroup encounters or severe anthropogenic disturbances could independently trigger both physiological stress responses and vocal modifications (Cañadas-Santiago et al., 2020; Cristóbal-Azkarate et al., 2007), potentially creating correlations without direct physiological links between hormones and vocal production. Similarly, seasonal variation in resource availability or social dynamics could affect both physiological stress levels and calling behavior through separate pathways (Kitchen et al., 2015). Methodological limitations in fGCM measurement, including the delay in glucocorticoid metabolite excretion (Goymann, 2012; Palme, 2019), could also introduce temporal mismatches between vocal recordings and hormonal measurements. The excretion lag time of approximately 20–30 h (Aguilar-Cucurachi et al., 2010) creates inherent uncertainty in matching vocal behavior to precise hormonal states. Future research should address these limitations through larger sample sizes with repeated measurements to account for individual variation and more precise temporal matching of vocal and hormonal samples (e.g., measuring glucocorticoid metabolites in urine, which are excreted quicker). Future studies could also examine whether bout duration or other measures of calling intensity relate to acoustic properties or fGCM levels, providing additional insight into the relationship between behavioral context and stress physiology.

The broader implications of these findings extend to both primate communication and non-invasive stress monitoring. Our findings suggest that in male howler monkeys, variation in roar structure is associated with physiological state, providing evidence that vocal cues can convey information about an individual's condition and potentially influence social interactions (Briefer, 2012; Charlton et al., 2007; Ey et al., 2007; Muir et al., 2025). Our findings with howler monkeys also parallel research in other primate species, including chimpanzees (*Pan troglodytes*: Slocombe and Zuberbühler, 2007), baboons (*Papio ursinus*: Fischer et al., 2001), and macaques (*Macaca* spp.: Gouzoules and Gouzoules, 2000), which demonstrate that vocalizations carry information about

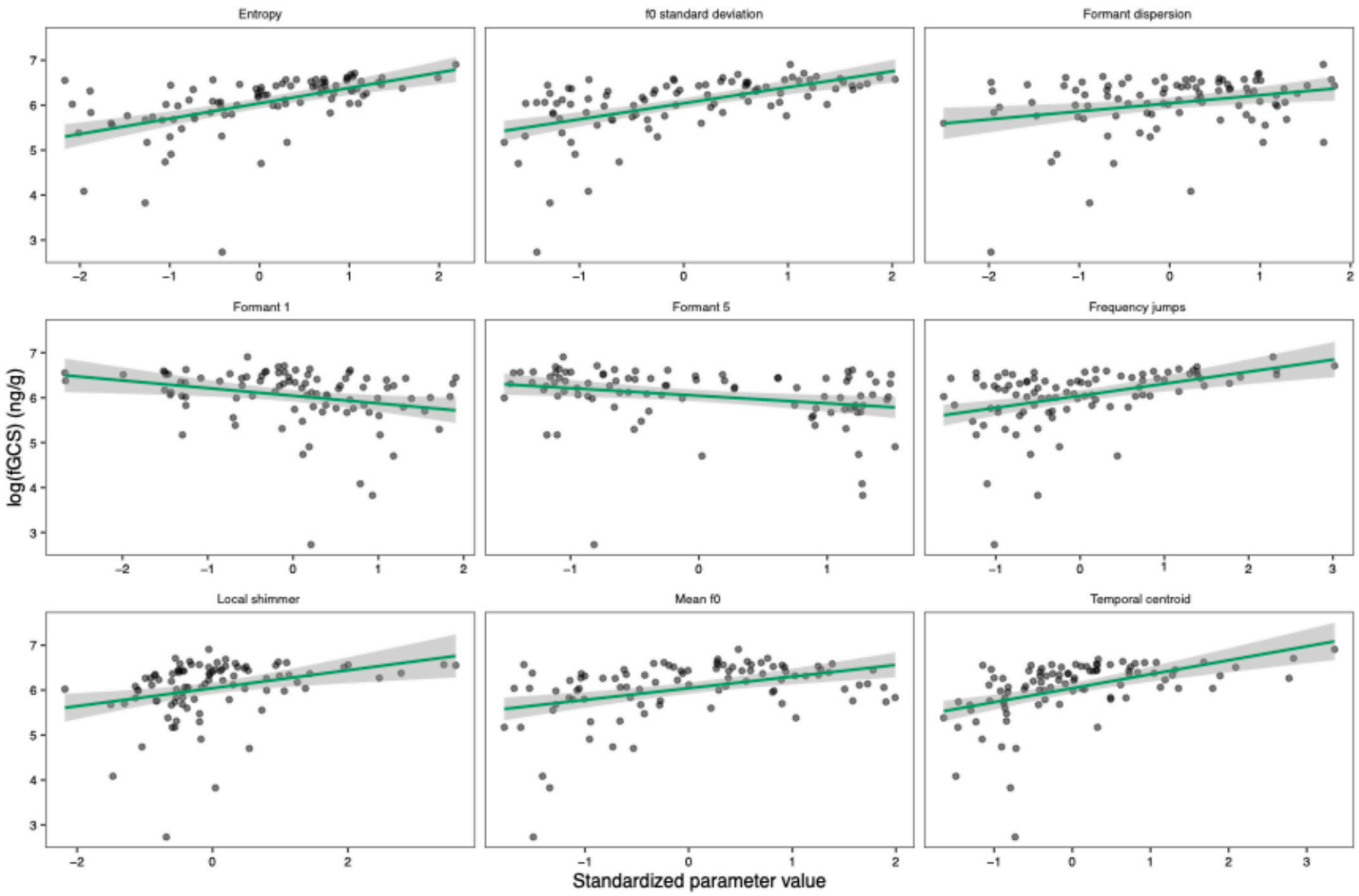

Fig. 4. Univariate relationships between the acoustic parameters of roar vocalizations produced by mantled howler monkey males and fecal glucocorticoid metabolite (fGCM) concentrations. Green lines show the predicted estimates from a linear mixed model and gray areas indicate its 95 % confidence intervals.

emotional states. However, howler monkeys' specialized vocal anatomy, including their enlarged hyoid bone (Dunn et al., 2015; Schön, 1971), may amplify or modify how stress manifests in their vocalizations compared to other primates. While the degree to which stress-induced vocal changes influence listener perception remains underexplored, previous research suggests that primates can discriminate between vocalizations encoding different emotional states (Seyfarth and Cheney, 2003), raising the possibility that these acoustic changes serve as honest signals that could influence social dynamics, including dominance interactions and mating decisions (Bercovitch and Ziegler, 2002; Creel et al., 2013; Taylor and Reby, 2010).

From a conservation and welfare perspective, our findings suggest that acoustic monitoring could provide a valuable complementary tool for non-invasive stress assessment in wild primates. Acoustic methods offer advantages in accessibility and continuous monitoring capabilities compared to traditional fecal hormone sampling (Blumstein et al., 2011; de la Torre and Snowdon, 2002; Kalan et al., 2015). In fragmented landscapes like Los Tuxtlas, where anthropogenic pressures create various stressors (Dias et al., 2017; Rangel-Negrín et al., 2021; Cristóbal-Azkarate et al., 2007), remote acoustic monitoring could help identify populations experiencing chronic stress, potentially informing conservation prioritization. However, given the complexity of the relationship between acoustic parameters and hormonal states, we recommend that acoustic monitoring be used alongside traditional endocrine methods, similar to approaches successfully implemented in other taxa (Nowacek et al., 2016).

In conclusion, our study provides evidence that howler monkey vocalizations reflect physiological stress, with significant correlations between fGCM and specific acoustic parameters, particularly mean f0, f0 range, and various perturbation measures including shimmer and jitter. These findings support the putative involvement of three physiological mechanisms through which physiological stress can affect vocalization: increased laryngeal muscle tension (revealed in f0 and perturbation measures), altered respiratory control (evident in temporal parameters), and modified vocal tract configuration (reflected in formant structure and spectral properties). These findings establish a link between stress physiology and vocal production in a wild primate, expanding our understanding of how internal states manifest in communicative signals (Endler, 1992). These results open new avenues for understanding primate communication from both evolutionary and mechanistic perspectives, while also offering potential applications for non-invasive welfare monitoring in contexts of habitat disturbance.

CRediT authorship contribution statement

Ariadna Rangel-Negrín: Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jacob C. Dunn:** Writing – original draft, Supervision, Formal analysis. **Alejandro Coyohua-Fuentes:** Writing – review & editing, Methodology, Investigation, Data curation. **David Roberto Chavira-Ramírez:** Writing – review & editing, Methodology, Investigation. **Pedro A.D. Dias:** Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

none.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2025.105840.

Data availability

Data and code used in this paper are available on figshare: doi: https://doi.org/10.6084/m9.figshare.28628543.

References

Aguilar-Cucurachi, M.S., Dias, P.A.D., Rangel-Negrín, A., Chavira, R., Boeck, L., Canales-Espinosa, D., 2010. Accumulation of stress during translocation in mantled howlers. Am. J. Primatol. 72 (8), 805–810. https://doi.org/10.1002/ajp.20841.

Alcocer-Rodríguez, M., Arroyo-Rodríguez, V., Galán-Acedo, C., Cristóbal-Azkarate, J., Asensio, N., Rito, K.F., Hawes, J.E., Veà Baró, J.J., Dunn, J.C., 2021. Evaluating extinction debt in fragmented forests: the rapid recovery of a critically endangered primate. Anim. Conserv. 24 (3), 432–444. https://doi.org/10.1111/acv.12648.

Baldwin, J.D., Baldwin, J.I., 1976. Vocalizations of howler monkeys (*Alouatta palliata*) in southwestern Panama. Folia Primatol. 26 (2), 81–108. https://doi.org/10.1159/000155733.

Bercovitch, F.B., Ziegler, T.E., 2002. Current topics in primate socioendocrinology. Annu. Rev. Anthropol. 31 (1), 45–67. https://doi.org/10.1146/annurev.anthro.31.040202.105553.

Bergman, T.J., Cortés-Ortiz, L., Dias, P.A.D., Ho, L., Adams, D., Canales-Espinosa, D., Kitchen, D.M., 2016. Striking differences in the loud calls of howler monkey sister species (*Alouatta pigra* and *a palliata*). Am. J. Primatol. 78 (7), 755–766. https://doi.org/10.1002/ajp.22539.

Blumstein, D.T., Chi, Y.Y., 2012. Scared and less noisy: glucocorticoids are associated with alarm call entropy. Biol. Lett. 8 (2), 189–192. https://doi.org/10.1098/rsbl.2011.0832.

Blumstein, D.T., Mennill, D.J., Clemins, P., Girod, L., Yao, K., Patricelli, G., Deppe, J.L., Krakauer, A.H., Clark, C., Cortopassi, K.A., Hanser, S.F., McCowan, B., Ali, A.M., Kirschel, A.N.G., 2011. Acoustic monitoring in terrestrial environments using microphone arrays: applications, technological considerations and prospectus. J. Appl. Ecol. 48 (3), 758–767. https://doi.org/10.1111/j.1365-2664.2011.01993.x.

Bradbury, J.W., Vehrencamp, S.L., 2011. Principles of Animal Communication, 2nd ed. Sinauer Associates.

Briefer, E.F., 2012. Vocal expression of emotions in mammals: mechanisms of production and evidence. J. Zool. 288 (1), 1–20. https://doi.org/10.1111/j.1469-7998.2012.00920.x.

Burnham, K.P., Anderson, D.R., 2013. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2nd ed. Springer Science Business Media.

Cañadas-Santiago, S., Dias, P.A.D., Garau, S., Coyohua-Fuentes, A., Chavira Ramírez, D. R., Canales-Espinosa, D., Rangel-Negrín, A., 2020. Behavioral and physiological stress responses to local spatial disturbance and human activities by howler monkeys at Los Tuxtlas. Mexico. Anim Conserv. 23 (3), 297–306. https://doi.org/10.1111/acv.12541.

Catchpole CK, Slater PJB. 2008. Bird Song: Biological Themes and Variations (2nd ed.). Cambridge University Press.

Ceccarelli, E., Rangel-Negrín, A., Coyohua-Fuentes, A., Canales-Espinosa, D., Dias, P.A. D., 2021. Vocal and movement responses of mantled howler monkeys (*Alouatta palliata*) to natural loud calls from neighbors. Am. J. Primatol. 83 (4), e23252. https://doi.org/10.1002/ajp.23252.

Charlton, B.D., Reby, D., McComb, K., 2007. Female red deer prefer the roars of larger males. Biol. Lett. 3 (4), 382–385. https://doi.org/10.1098/rsbl.2007.0244.

Chivers DJ. 1969. On the daily behaviour and spacing of howling monkey groups. Folia Primatol. 10(1–2): 48–102. doi:https://doi.org/10.1159/000155188.

Coudé, G., Ferrari, P.F., Rodà, F., Maranesi, M., Borelli, E., Veroni, V., Monti, F., Rozzi, S., Fogassi, L., 2011. Neurons controlling voluntary vocalization in the macaque ventral premotor cortex. PLoS One 6 (11), e26822. https://doi.org/10.1371/journal.pone.0026822.

Creel, S., Dantzer, B., Goymann, W., Rubenstein, D.R., 2013. The ecology of stress: effects of the social environment. Funct. Ecol. 27 (1), 66–80. https://doi.org/10.1111/j.1365-2435.2012.02029.x.

Cristóbal-Azkarate, J., Chavira, R., Boeck, L., Rodríguez-Luna, E., Veà, J.J., 2007. Glucocorticoid levels in free-ranging resident mantled howlers: a study of coping strategies. Am. J. Primatol. 69 (8), 866–876. https://doi.org/10.1002/ajp.20383.

Cristóbal-Azkarate, J., Dunn, J.C., Balcells, C.D., Veà, J.J., 2017. A demographic history of a population of howler monkeys (*Alouatta palliata*) living in a fragmented landscape in Mexico. PeerJ 5, e3547. https://doi.org/10.7717/peerj.3547.

da Cunha, R.G.T., de Oliveira, D.A.G., Holzmann, I., Kitchen, D.M., 2015. Production of loud and quiet calls in howler monkeys. In: Kowalewski, M.M., Garber, P.A., Cortés-Ortiz, L., Urbani, B., Youlatos, D. (Eds.), Howler Monkeys: Adaptive Radiation, Systematics, and Morphology. Springer, pp. 337–368.

de la Torre, S., Snowdon, C.T., 2002. Environmental correlates of vocal communication of wild pygmy marmosets, *Cebuella pygmaea*. Anim. Behav. 63 (5), 847–856. https://doi.org/10.1006/anbe.2001.1978.

Di Fiore, A., Campbell, C.J., 2007. The atelines: Variation in ecology, behavior, and social organization. In: Campbell, C.J., Fuentes, A., MacKinnon, K.C., Panger, M., Bearder, S.K. (Eds.), Primates in Perspective. Oxford University Press, pp. 155–185.

Dias, P.A.D., Coyohua-Fuentes, A., Canales-Espinosa, D., Chavira Ramírez, D.R., Rangel-Negrín, A., 2017. Hormonal correlates of energetic condition in mantled howler monkeys. Horm. Behav. 94, 13–20. https://doi.org/10.1002/ajpa.24464.

Dias, P.A.D., Coyohua-Fuentes, A., Canales-Espinosa, D., Chavira Ramírez, D.R., Rangel-Negrín, A., 2022. Correlates of hormonal variation in mantled howler monkey males. Alouatta palliata. Am J Phys Anthropol. 178 (1), 17–28. https://doi.org/10.1002/ajpa.24464.

Dirzo, R., García, MC., 1992. Rates of deforestation in Los Tuxtlas, a neotropical area in Southern Mexico. Conserv. Biol. 6 (1), 84–90.

Dunn, J.C., Cristóbal-Azkarate, J., Chavira, R., Veà, J.J., 2013. Travel time predicts fecal glucocorticoid levels in free-ranging howlers (*Alouatta palliata*). Int. J. Primatol. 34 (2), 246–259. https://doi.org/10.1007/s10764-013-9657-0.

Dunn, J.C., Halenar, L.B., Davies, T.G., Cristóbal-Azkarate, J., Reby, D., Sykes, D., Dengg, S., Fitch, W.T., Knapp, L.A., 2015. Evolutionary trade-off between vocal tract and testes dimensions in howler monkeys. Curr. Biol. 25 (21), 2839–2844. https://doi.org/10.1016/j.cub.2015.09.029.

Endler, J.A., 1992. Signals, signal conditions, and the direction of evolution. Am. Nat. 139, S125–S153. https://doi.org/10.1086/285308.

Ey, E., Pfefferle, D., Fischer, J., 2007. Do age- and sex-related variations reliably reflect body size in non-human primate vocalizations? A review. Primates 48 (4), 253–267. https://doi.org/10.1007/s10329-006-0033-y.

Fischer, J., Metz, M., Cheney, D.L., Seyfarth, R.M., 2001. Baboon responses to graded bark variants. Anim. Behav. 63 (1), 105–111. https://doi.org/10.1006/anbe.2000.1687.

Fitch, W.T., Hauser, M.D., 2002. Unpacking "honesty": Vertebrate vocal production and the evolution of acoustic signals. In: Simmons, A.M., Popper, A.N., Fay, R.R. (Eds.), Acoustic Communication. Springer, pp. 65–137.

Freeberg, T.M., Dunbar, R.I.M., Ord, T.J., 2012. Social complexity as a proximate and ultimate factor in communicative complexity. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 367 (1597), 1785–1801. https://doi.org/10.1098/rstb.2011.0213.

Gerhardt, H.C., Huber, F., 2002. Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. University of Chicago Press.

Giddens, C.L., Barron, K.W., Byrd-Craven, J., Clark, K.F., Winter, A.S., 2013. Vocal indices of stress: a review. J. Voice 27 (3), 390.e21–390.e29. https://doi.org/10.1016/j.jvoice.2012.12.010.

Gómez-Espinosa, E., Dias, P.A.D., Rangel-Negrín, A., 2022. The influence of anthropogenic noise on the behavior of male mantled howler monkeys. Am. J. Primatol. 84 (5), e23377. https://doi.org/10.1002/ajp.23377.

Gouzoules, H., Gouzoules, S., 2000. Agonistic screams differ among four species of macaques: the significance of motivation-structural rules. Anim. Behav. 59 (3), 501–512. https://doi.org/10.1006/anbe.1999.1318.

Goymann, W., 2012. On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. Methods Ecol. Evol. 3 (4), 757–765. https://doi.org/10.1111/j.2041-210X.2012.00203.x.

Hage, S.R., Nieder, A., 2013. Single neurons in monkey prefrontal cortex encode volitional initiation of vocalizations. Nat. Commun. 4 (1), 2409. https://doi.org/10.1038/ncomms3409.

Hammerschmidt, K., Fischer, J., 2008. Constraints in primate vocal production. In: Oller, D.K., Griebel, U. (Eds.), Evolution of Communicative Flexibility: Complexity, Creativity, and Adaptability in Human and Animal Communication. MIT Press, pp. 93–119.

Herbst CT, Tokuda IT, Nishimura T. Ternström, S, Ossio V, Levy M, Fitch WT, Dunn JC. 2025. 'Monkey yodels'—frequency jumps in New World monkey vocalizations greatly surpass human vocal register transitions. Philos Trans R Soc B Biol Sci. 380: 20240005. doi:https://doi.org/10.1098/rstb.2024.0005, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.

Hopkins, M.E., 2013. Relative dominance and resource availability mediate mantled howler (*Alouatta palliata*) spatial responses to neighbors' loud calls. Int. J. Primatol. 34 (5), 1032–1054. https://doi.org/10.1007/s10764-013-9713-9.

Johnson, K., 2020. The ΔF method of vocal tract length normalization for vowels. Lab Phonol. 11 (1), 1–16. https://doi.org/10.5334/labphon.196.

Jürgens, U., 2009. The neural control of vocalization in mammals: a review. J. Voice 23 (1), 1–10. https://doi.org/10.1016/j.jvoice.2007.07.005.

Kalan AK, Mundry R, Wagner OJ J, Heinicke S, Boesch C. Kühl, HS. 2015. Towards the automated detection and occupancy estimation of primates using passive acoustic monitoring. Ecol. Indic. 54: 217–226. doi:https://doi.org/10.1016/j.ecolind.2015.02.023.

Kikusui, T., Winslow, J.T., Mori, Y., 2011. Social buffering: relief from stress and anxiety. Philos Trans R Soc B Biol Sci. 366 (1571), 2215–2228. https://doi.org/10.1098/rstb.2006.1941.

Kitchen, D.M., da Cunha RG T, Holzmann, I., Oliveira DA G, 2015. Function of loud calls in howler monkeys. In: Kowalewski, M.M., Garber, P.A., Cortés-Ortiz, L., Urbani, B., Youlatos, D. (Eds.), Howler Monkeys: Adaptive Radiation, Systematics, and Morphology. Springer, pp. 369–399.

Koren, L., Mokady, O., Geffen, E., 2008. Social status and cortisol levels in singing rock hyraxes. Horm. Behav. 54 (1), 212–216. https://doi.org/10.1016/j.yhbeh.2008.02.020.

Linhart, P., Ratcliffe, V.F., Reby, D., Špinka, M., 2015. Expression of emotional arousal in two different piglet call types. PLoS One 10 (8), e0135414. https://doi.org/10.1371/journal.pone.0135414.

Maya-Lastra, N., Dias, P.A.D., Chavira-Ramírez, D.R., Rangel-Negrín, A., 2025. "dear enemies", "nasty neighbors", and the strength in numbers: exploring the behavioral and hormonal responses of mantled howler monkey males to simulated intruder loud calls. Am. J. Primatol. 97, e70032. https://doi.org/10.1002/ajp.70032.

McGregor, P.K., 2005. Animal Communication Networks. Cambridge University Press.

Muir J, Herbst CT, Hawes JE O'Mahoney T, Dunn JC. 2025. Nonlinear phenomena in mammalian vocal communication: an introduction and scoping review. Philos Trans R Soc B Biol Sci. 380: 20240017. doi:https://doi.org/10.1098/rstb.2024.0017, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.

Mukudai, S., Matsuda, K.I., Nishio, T., Sugiyama, Y., Bando, H., Hirota, R., Uemura, T., Muranaka, T., Yamashita, M., Kawata, M., 2015. Differential responses to steroid hormones in fibroblasts from the vocal fold, trachea, and esophagus. Endocrinology 156 (3), 1000–1009. https://doi.org/10.1210/en.2014-1605.

Nowacek, D.P., Christiansen, F., Bejder, L., Goldbogen, J.A., Friedlaender, A.S., 2016. Studying cetacean behaviour: new technological approaches and conservation applications. Anim. Behav. 120, 235–244. https://doi.org/10.1016/j.anbehav.2016.07.019.

Nowicki, S., Searcy, W.A., 2004. Song function and the evolution of female preferences: why birds sing, why brains matter. Ann. N. Y. Acad. Sci. 1016 (1), 704–723. https://doi.org/10.1196/annals.1298.012.

Owren, M.J., Rendall, D., 2003. Salience of caller identity in rhesus monkey (*Macaca mulatta*) coos and screams: perceptual experiments with human listeners. J. Comp. Psychol. 117 (4), 380–390. https://doi.org/10.1037/0735-7036.117.4.380.

Palme, R., 2019. Non-invasive measurement of glucocorticoids: advances and problems. Physiol. Behav. 199, 229–243. https://doi.org/10.1016/j.physbeh.2018.11.021.

Pisanski, K., Sorokowski, P., 2020. Human stress detection: cortisol levels in stressed speakers predict voice-based judgments of stress. Perception 50 (1), 80–87. https://doi.org/10.1177/0301006620978.

Dias PAD, Gómez-Espinosa EE, Chavira-Ramírez R, Rangel-Negrín A. 2023. Noise intensity modulates the responses of mantled howler monkeys to anthropophony. Am. J. Primatol. 85(9): e23568. doi:https://doi.org/10.1002/ajp.23568.

Rangel-Negrín, A., Coyohua-Fuentes, A., de la Torre Herrera, A., Cano-Huertes, B., Reynoso-Cruz, E., Ceccarelli, E., Gómez Espinosa, E., Chavira Ramírez, D.R., Moreno-Espinoza, D., Canales-Espinosa, D., Maya-Lastra, N., Cruz-Miros, P., Cañadas Santiago, S., Garau, S., Dias, P.A.D., 2021. Female reproductive energetics in mantled howler monkeys (*Alouatta palliata*): a follow-up study. Am. J. Phys. Anthropol. 174 (3), 396–406. https://doi.org/10.1002/ajpa.24222.

Rangel-Negrín, A., Gómez-Espinosa, E., Chavira-Ramírez, D.R., Dias, P.A.D., 2023. Dog barks influence the physiological stress and behavior of a wild primate species. Sci. Total Environ. 875, 163585. https://doi.org/10.1016/j.scitotenv.2023.163585.

Reby, D., McComb, K., 2003. Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. Anim. Behav. 65, 519–530. https://doi.org/10.1006/anbe.2003.2078.

Riede, T., Goller, F., 2010. Peripheral mechanisms for vocal production in birds - differences and similarities to human speech and singing. Brain Lang. 115 (1), 69–80. https://doi.org/10.1016/j.bandl.2009.11.003.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21 (1), 55–89. https://doi.org/10.1210/edrv.21.1.0389.

Scherer, K.R., 2003. Vocal communication of emotion: a review of research paradigms. Speech Comm. 40 (1–2), 227–256. https://doi.org/10.1016/S0167-6393(02)00084-5.

Scherer, K.R., 2009. The dynamic architecture of emotion: evidence for the component process model. Cognit. Emot. 23 (7), 1307–1351. https://doi.org/10.1080/02699930902928969.

Schön, M.A., 1964. Possible function of some pharyngeal and lingual muscles of the howling monkey (*Alouatta seniculus*). Acta Anat. 58, 271–283. https://doi.org/10.1159/000142587.

Schön MA. 1971. The anatomy of the resonating mechanism in howling monkeys. Folia Primatol. 15(1–2): 117–132. doi:https://doi.org/10.1159/000155371.

Schrader, L., Todt, D., 1998. Vocal quality is correlated with levels of stress hormones in domestic pigs. Ethology 104, 859–876. https://doi.org/10.1111/j.1439-0310.1998.tb00036.x.

Sehrsweeney, M., Wilson, D.R., Bain, M., Boutin, S., Lane, J.E., McAdam, A.G., Dantzer, B., 2019. The effects of stress and glucocorticoids on vocalizations: a test in north American red squirrels. Behav. Ecol. 30, 1030–1040. https://doi.org/10.1093/beheco/arz044.

Seyfarth, R.M., Cheney, D.L., 2003. Signalers and receivers in animal communication. Annu. Rev. Psychol. 54 (1), 145–173. https://doi.org/10.1146/annurev.psych.54.101601.145121.

Slabbekoorn, H., Smith, T.B., 2002. Habitat-dependent song divergence in the little greenbul: an analysis of environmental selection pressures on acoustic signals. Evolution 56 (9), 1849–1858. https://doi.org/10.1111/j.0014-3820.2002.tb00199.x.

Slocombe, K.E., Zuberbühler, K., 2007. Chimpanzees modify recruitment screams as a function of audience composition. Proc. Natl. Acad. Sci. USA 104 (43), 17228–17233. https://doi.org/10.1073/pnas.0706741104.

Taylor, A.M., Reby, D., 2010. The contribution of source-filter theory to mammal vocal communication research. J. Zool. 280 (3), 221–236. https://doi.org/10.1111/j.1469-7998.2009.00661.x.

Team, Audacity, 2024. Audacity® (version 3.7.1) [computer software]. https://audacityteam.org/.

Titze, I.R., 1994. Principles of Voice Production. Prentice Hall.

Titze, I.R., Abbott, K.V., 2012. Vocology: The Science and Practice of Voice Habilitation. National Center for Voice and Speech.

Tolkmitt, F.J., Scherer, K.R., 1986. Effects of experimentally induced stress on vocal parameters. J. Exp. Psychol. Hum. Percept. Perform. 12, 302–313. https://doi.org/10.1037/0096-1523.12.3.302.

Viljoen, J.J., Ganswindt, A., Reynecke, C., Stoeger, A.S., Langbauer, W.R., 2014. Vocal stress associated with a translocation of a family herd of African elephants (*Loxodonta africana*) in the Kruger National Park. South Africa. Bioacoustics. 24 (1), 1–12. https://doi.org/10.1080/09524622.2014.906320.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120 (3), 260–275. https://doi.org/10.1006/gcen.2000.7557.

Whitehead, J.M., 1987. Vocally mediated reciprocity between neighbouring groups of mantled howling monkeys, *Alouatta palliata palliata*. Anim. Behav. 35 (6), 1615–1627. https://doi.org/10.1016/S0003-3472(87)80054-4.

Wich, S.A., Nunn, C.L., 2002. Do male "long-distance calls" function in mate defense? A comparative study of long-distance calls in primates. Behav. Ecol. Sociobiol. 52 (6), 474–484. https://doi.org/10.1007/s00265-002-0541-8.

Wiley, R.H., Richards, D.G., 1978. Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. Behav. Ecol. Sociobiol. 3 (1), 69–94. https://doi.org/10.1007/BF00300047.